ANNALS OF BOTANY

EDITED BY

DUKINFIELD HENRY SCOTT, M.A., LL.D., D.Sc., F.R.S.
LATELY HONORARY KEEPER OF THE JODRELL LABORATORY, ROYAL BOTANIC GARDENS, KEW

JOHN BRETLAND FARMER, D.Sc., M.A., F.R.S.
PROFESSOR OF BOTANY, ROYAL COLLEGE OF SCIENCE, LONDON

FRANCIS WALL OLIVER, M.A., D.Sc., F.R.S.
QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

AND

ROLAND THAXTER, M.A., Ph.D.
PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

ASSISTED BY OTHER BOTANISTS

VOLUME XXXIII

With Twenty-eight Plates and One Hundred and Eighty-eight Figures in the Text

LONDON
HUMPHREY MILFORD, OXFORD UNIVERSITY PRESS
AMEN CORNER, E.C.
EDINBURGH, GLASGOW, NEW YORK, TORONTO, MELBOURNE, AND BOMBAY
1919

PRINTED IN ENGLAND
AT THE OXFORD UNIVERSITY PRESS

CONTENTS.

PAGE

WILLIAM GIBSON FARLOW . . . . . xv–xvi

No. CXXIX, January, 1919.

SCOTT, D. H.—On the Fertile Shoots of Mesoxylon and an Allied Genus. With Plates I–III and three Figures in the Text 1

WILLIS, J. C.—The Flora of Stewart Island (New Zealand) : a Study in Taxonomic Distribution. With two Maps and fourteen Tables in the Text 23

SALISBURY, E. J.—Variation in Eranthis hyemalis, Ficaria verna, and other Members of the Ranunculaceae, with Special Reference to Trimery and the Origin of the Perianth. With twenty Figures in the Text and Tables I–X 47

SAHNI, B.—On an Australian Specimen of Clepsydropsis. With Plate IV and two Figures in the Text 81

BRACHER, ROSE.—Observations on Euglena deses. With nine Figures in the Text . . 93

STEIL, W. N.—A Study of Apogamy in Nephrodium hirtipes, Hk. With Plates V–VII . 103

NOTE.

GROOM, PERCY.—The Wood of Tetracentron, Trochodendron, Drimys, and other Types . 133

No. CXXX, April, 1919.

DIGBY, L.—On the Archesporial and Meiotic Mitoses of Osmunda. With Plates VIII–XII and thirty-five Figures in the Text 135

ARBER, the late E. A. NEWELL.—Remarks on the Organization of the Cones of Williamsonia gigas (L. and H.). With five Figures in the Text 173

HAINES, F. M.—A New Auxanometer. With two Figures in the Text 181

SPRATT, ETHEL R.—A Comparative Account of the Root-nodules of the Leguminosae. With Plate XIII and five Figures in the Text 189

TUTTLE, GWYNETHE M.—Induced Changes in Reserve Materials in Evergreen Herbaceous Leaves. With seven Figures in the Text 201

ARBER, AGNES.—On the Law of Age and Area, in Relation to the Extinction of Species . 211

CARTER, NELLIE.—Studies on the Chloroplasts of Desmids. I. With Plates XIV–XVIII . 215

HOLMES, M. G.—Observations on the Anatomy of Ash-wood with Reference to Water-conductivity. With seven Figures in the Text 253

NOTE.

PHILLIPS, R. W.—Note on the Duration of the Prothallia of Lastraea Filix-mas, Presl. . . 265

No. CXXXI, July, 1919.

WILLIS, J. C.—The Floras of the Outlying Islands of New Zealand and their Distribution. With two Maps and twenty-one Tables in the Text 267

CARTER, N.—Studies on the Chloroplasts of Desmids. II. With Plates XIX and XX and one Figure in the Text 295

DEY, P. K.—Studies in the Physiology of Parasitism. V. Infection by Colletotrichum Lindemuthianum. With Plate XXI 305

WHITBY, STAFFORD.—Variation in Hevea brasiliensis. With one Diagram in the Text . 313

CLELAND, RALPH E.—The Cytology and Life-history of Nemalion multifidum, Ag. With Plates XXII–XXIV and three Figures in the Text 323

BLACKMAN, V. H.—The Compound Interest Law and Plant Growth 353

WORMALD, H.—The 'Brown Rot' Diseases of Fruit Trees, with Special Reference to two Biologic Forms of Monilia cinerea, Bon. I. With Plates XXV and XXVI . . 361

PAGE

No. CXXXII, October, 1919.

PETCH, T.—Mocharas and the Genus Haematomyces. With two Figures in the Text . . 495
WORSDELL, W. C.—The Origin and Meaning of Medullary (Intraxylary) Phloem in the Stems of Dicotyledons. II. Compositae. With twenty-seven Figures in the Text . 421
ARBER, AGNES.—Studies on Intrafascicular Cambium in Monocotyledons (III and IV). With seven Figures in the Text 459
CARTER, NELLIE.—The Cytology of the Cladophoraceae. With Plate XXVII and two Figures in the Text 467
WILLIS, J. C.—On the Floras of Certain Islets outlying from Stewart Island (New Zealand). With one Map in the Text 479
OSBORN, T. G. B.—Some Observations on the Tuber of Phylloglossum. With Plate XXVIII and forty-three Figures in the Text 485
SMITH, A. MALINS.—The Temperature-coefficient of Photosynthesis: a Reply to Criticism. With two Figures in the Text 517

INDEX.

A. ORIGINAL PAPERS AND NOTES.

PAGE

ARBER, AGNES.—On the Law of Age and Area, in Relation to the Extinction of Species . 211

——————— Studies on Intrafascicular Cambium in Monocotyledons (III and IV). With seven Figures in the Text 459

ARBER, the late E. A. NEWELL.—Remarks on the Organization of the Cones of Williamsonia gigas (L. and H.). With five Figures in the Text 173

BLACKMAN, V. H.—The Compound Interest Law and Plant Growth 353

BRACHER, ROSE.—Observations on Euglena deses. With nine Figures in the Text . . 93

CARTER, NELLIE.—Studies on the Chloroplasts of Desmids. I. With Plates XIV–XVIII . 215

——————— Studies on the Chloroplasts of Desmids. II. With Plates XIX and XX and one Figure in the Text 295

——————— The Cytology of the Cladophoraceae. With Plate XXVII and two Figures in the Text 467

CLELAND, RALPH E.—The Cytology and Life-history of Nemalion multifidum, Ag. With Plates XXII–XXIV and three Figures in the Text 323

DEY, P. K.—Studies in the Physiology of Parasitism. V. Infection by Colletotrichum Lindemuthianum. With Plate XXI 305

DIGBY, L.—On the Archesporial and Meiotic Mitoses of Osmunda. With Plates VIII–XII and thirty-five Figures in the Text 135

GROOM, PERCY.—The Wood of Tetracentron, Trochodendron, Drimys, and other Types . 133

HAINES, F. M.—A New Auxanometer. With two Figures in the Text 181

HOLMES, M. G.—Observations on the Anatomy of Ash-wood with Reference to Water-conductivity. With seven Figures in the Text 255

Obituary :

WILLIAM GIBSON FARLOW . . . . . xv–xvi

OSBORN, T. G. B.—Some Observations on the Tuber of Phylloglossum. With Plate XXVIII and forty-three Figures in the Text 485

PETCH, T.—Mocharas and the Genus Haematomyces. With two Figures in the Text . . 405

PHILLIPS, R. W.—Note on the Duration of the Prothallia of Lastraea Filix-mas, Presl. . . 265

SAHNI, B.—On an Australian Specimen of Clepsidropsis. With Plate IV and two Figures in the Text 81

SALISBURY, E. J.—Variation in Eranthis hyemalis, Ficaria verna, and other Members of the Ranunculaceae, with Special Reference to Trimery and the Origin of the Perianth. With twenty Figures in the Text and Tables I–X 47

SCOTT, D. H.—On the Fertile Shoots of Mesoxylon and an Allied Genus. With Plates I–III and three Figures in the Text 1

SMITH, A. MALINS.—The Temperature-coefficient of Photosynthesis: a Reply to Criticism. With two Figures in the Text 517

SPRATT, ETHEL R.—A Comparative Account of the Root-nodules of the Leguminosae. With Plate XIII and five Figures in the Text 189

STEIL, W. N.—A Study of Apogamy in Nephrodium hirtipes, Hk. With Plates V–VII . 109

TUTTLE, GWYNETHE M.—Induced Changes in Reserve Materials in Evergreen Herbaceous Leaves. With seven Figures in the Text 201

WHITBY, STAFFORD.—Variation in Hevea brasiliensis. With one Diagram in the Text . . 313

WILLIS, J. C.—The Flora of Stewart Island (New Zealand): a Study in Taxonomic Distribution. With two Maps and fourteen Tables in the Text 23

PAGE

WILLIS, J. C.—The Floras of the Outlying Islands of New Zealand and their Distribution. With two Maps and twenty-one Tables in the Text 267

———— On the Floras of Certain Islets outlying from Stewart Island (New Zealand). With one Map in the Text 479

WORMALD, H.—The 'Brown Rot' Diseases of Fruit Trees, with Special Reference to two Biologic Forms of Monilia cinerea, Bon. I. With Plates XXV and XXVI . . 361

WORSDELL, W. C.—The Origin and Meaning of Medullary (Intraxylary) Phloem in the Stems of Dicotyledons. II. Compositae. With twenty-seven Figures in the Text . . 421

B. LIST OF ILLUSTRATIONS.

a. PLATES.

I. Mesoxylon and Mesoxylopsis (SCOTT).
II. Mesoxylopsis and Mitrospermum (SCOTT).
III. Mesoxylon (SCOTT).
IV. Clepsydropsis (SAHNI).
V–VII. Nephrodium hirtipes (STEIL).
VIII–XII. Mitoses in Osmunda (DIGBY).
XIII. Leguminous Root-nodules (SPRATT).
XIV–XVIII. Chloroplasts of Desmids (CARTER).
XIX, XX. Micrasterias (CARTER).
XXI. Colletotrichum (DEY).
XXII–XXIV. Nemalion (CLELAND).
XXV, XXVI. Monilia (WORMALD).
XXVII. Cladophoraceae (CARTER).
XXVIII. Phylloglossum (OSBORN).

. FIGURES.

1. Transverse section of fertile shoot, A, and its branch, B (SCOTT) . . 2
2. Transverse section of fertile shoot, A, and bracts, B. At B, two bracts, belonging to a branch of the shoot (SCOTT) 5
3. Part of a branch of Cordaites laevis (restored). It bears several leaves and two inflorescences, the upper ♀, the lower ♂, with distichous floral buds. From Grand'Eury (SCOTT) 9
Map of New Zealand and outlying islands (WILLIS) 25
" " " " " 26
1. Flower of Eranthis hyemalis. The roman numerals indicate the order of dehiscence (SALISBURY) 48
2. Eranthis hyemalis. A and B, flowers with the androecium dissected away, showing the paired character of the supernumerary honey-leaves (*φ*); C, floral diagram of typical cyclic flower; D, partially bifurcated honey-leaf; E and F, petaloid honey-leaves; G and H, honey-leaves bearing anthers (*α*); I and J, branched stamens; K, scar of outer perianth segment; L, of inner perianth segment; M, scar of nectary; N, scar of stamen; *v.b.* vascular strand (SALISBURY) . 49
3. Ditto. Empirical diagrams showing arrangement of honey-leaves and stamens (SALISBURY) 51
4. Variation 'curves' for perianth, honey-leaves, and gynaeceum of E. hyemalis (SALISBURY) 52
5. Eranthis hyemalis. A and B, lobed perianth segments; C, trilobed bract replacing a perianth member; D, partially virescent perianth segment; E–G, lobed perianth segments; H, partially coloured bract; I, supernumerary perianth segment (SALISBURY) 53

PAGE

JRES. 6. Ditto. Empirical diagrams showing arrangement of bracts and perianth segments in abnormal flowers (SALISBURY) 54
7. Ditto. Meristic variation in androecium (SALISBURY) . . . 55
8. Fused carpel and stamen of Eranthis hyemalis (A) and branched carpel of Helleborus niger (B) (SALISBURY) 56
9. Eranthis hyemalis. Variation in bracts (SALISBURY) . . . 58
10. Ficaria verna. Empirical diagrams showing arrangement of the perianth segments and stamens (SALISBURY) 62
11. A and B, branched stamens of Ficaria verna; C, petaloid stamen; D, partially coloured sepal; E, sepal with lobed lamina; F, Anemone nemorosa, branched stamen; G, bilobed petal of Ficaria verna; H–J, variation in the sepals of F. verna (SALISBURY) . . . 63
12. Ficaria verna. Variation 'curve' for androecium (SALISBURY) . . 64
13. Ditto. Variation 'curve' for gynaeceum (SALISBURY) . . . 65
14. Anemone nemorosa. Variation 'curve' for androecium (SALISBURY) 68
15. Ditto. Variation 'curve' for gynaeceum (SALISBURY) . . . 68
16. A, diagram of perianth of A. nemorosa; B, floral diagram of Aconitum napellus; C, branched stamen of Aquilegia sp.; D and E, branched stamens of Delphinium sp.; F, diagram of perianth of Ranunculus bulbosus (SALISBURY) 69
17. Anemone napellus. Variation 'curve' for androecium (SALISBURY) . 70
18. Variation 'curve' for the androecium of Aconitum Lycoctonum (SALISBURY) 70
19. Variation 'curve' for the gynaeceum of Aquilegia sp. (SALISBURY) . 72
20. Stamens of Ranunculus auricomus, var. depauperata, developed in place of the petals (SALISBURY) 73
1. Diagram to show the fossil in plan and elevation (SAHNI) . . . 85
2. Ditto illustrating the extent to which the intruded roots distort the normal clepsydroid shape of the leaf-traces (SAHNI) . . . 87
1. Euglena deses, showing changes in shape occurring in one individual during several minutes' observation (BRACHER) 95
2. E. deses. Diurnal differences in appearance. (1) Early morning (actively moving); (2) midday (lying still on mud surface); (3) night (rounded off) (BRACHER) 96
3. Apparatus for producing tidal effect over area of Avon mud (BRACHER) 97
4. Graph representing the intensity of greenness on the mud under normal conditions with reference to the periods of high water and of darkness (BRACHER) 98
5. A graph drawn to illustrate the behaviour of E. deses when removed from the influence of the tide (BRACHER) 100
6. (1) Position of Euglenae at commencement of experiment: A, stiff mud; B, wet mud; C, mud covered by water; D, water only. (2) Position of Euglenae after five days' illumination from one side (BRACHER) 102
7. E. deses. 1–5, changes in shape of one individual when warmed from 1·5° C. to 16° C. (BRACHER) 103
8. Graph showing the time taken for Euglena deses to respond to the stimulus of light at different temperatures (BRACHER) . . . 104
9. Showing position taken up by Euglenae on the mud during rain (BRACHER) 105
1–8. Archesporial divisions. 9–12. Last archesporial division from metaphase. 13. Rest between last archesporial division and heterotype prophase. 14. Intermediate stage between the telophase of the last archesporial division and the heterotype prophase. 15–27. Heterotype division. 28–34. Homotype division. 35. Resting stage of tetrad (DIGBY) . . . . . *facing* 138

PAGE

FIGURES.

1. Restoration of female cone of Williamsonia gigas with the front bracts removed. 2. The same in section (E. A. N. ARBER) . . . 175
3. Restoration of male cone of Williamsonia gigas. 4. The same in section (E. A. N. ARBER) 176
5. Outline of the 'pyriform axis' (androphore) of a male cone of Williamsonia gigas (after W. C. Williamson) (E. A. N. ARBER) . . 177
1. A New Auxanometer (HAINES) 182
2. ,, ,, (HAINES) 184
1. *a*. Longitudinal section of root and young nodule of Alnus. *b*. Transverse section of nodule of Alnus. *c*. Longitudinal section of part of old branched nodule of Alnus (SPRATT) 190
2. *a*. Longitudinal section of nodule and root of Lupin. *b*. Longitudinal section of an older nodule (SPRATT) 192
3. Longitudinal section of nodule and root of Spartium (SPRATT) . . 193
4. *a*. Longitudinal section of nodule and root of Phaseolus. *b*. Longitudinal section of old nodule and root of Lotus corniculatus (SPRATT) . 193
5. Longitudinal section of nodule and root of Sophora (SPRATT) . . 195
1. Cross-section of Linnaea leaf, showing starch in material exposed to higher temperature for one week. Section treated with chloral hydrate and iodine (TUTTLE) 204
2. Horizontal section of Linnaea leaf, showing starch in epidermal cells. The material had been exposed to higher temperature for one week. Section treated with chloral hydrate and iodine (TUTTLE) . . 204
3. Horizontal section of Linnaea leaf, showing oils and fat in epidermal cells. Material fresh from outside before exposure to higher temperature. Section treated with 1 per cent. osmic acid (TUTTLE) . . 205
4. Linnaea material exposed to higher temperature for four days, sections of which were treated with chloral hydrate and iodine (TUTTLE) . 205
5. Cross-section of Linnaea leaf, showing fats and oils before exposure to higher temperature. Section treated with 1 per cent. osmic acid (TUTTLE) 206
6. Cross-section of Linnaea leaf, showing decreased fat and oil content. Material exposed to higher temperature for one week. Section treated with 1 per cent. osmic acid (TUTTLE) 206
7. Cross-section of Linnaea leaf. Material outside for reconversion during extremely low temperature. Section treated with iodine solution (TUTTLE) 207

Observations on the Anatomy of Ash-wood with reference to Water-conductivity (HOLMES)—

1. Specimens A 8, A 8 *a*, A 8 *b*, A 8 *e* (HOLMES) 256
2. Specimens A 3, A 4, A 6, A 8 *a*, A 8 *b*, A 8 *e* (HOLMES) . . . 257
3. Specimens A 3, A 4, A 6 (HOLMES) 257
4. Specimen A 3 (HOLMES) 258
5. Specimen A 4 (HOLMES) 259
6. Specimen A 6 (HOLMES) 260
7. Specimens A 8, A 8 *a*, A 8 *b*, A 8 *e* (HOLMES) 261

Map of New Zealand and outlying islands (WILLIS) 267

Map of New Zealand and islands to show imaginary distribution from Auckland and Dunedin (WILLIS) 268

Micrasterias apiculata, (Ehrenb.) Menegh (CARTER) 300

Diagram showing frequency curve and yield in grammes per day of 1,011 seven-year-old Hevea brasiliensis (WHITBY) . . . 317

1. The growing apex of the thallus. *a*. Apical cell of a young assimilative branch. *b*. Lateral budding to form a new branch. *c*. Terminal cell of a central strand filament (CLELAND) 325
2. Method of growth in a vegetative tuft. *a*. Lateral budding to form

FIGURES.		PAGE
	a side-branch. *b*. Terminal budding to form a trichome. *c*. False appearance of dichotomy. *d*. Trichome developed on the median line. *e*. Trichome developed to one side of the median line. *f*. Looped central strand filament (CLELAND)	326
3.	A cystocarp developing its first carpospores, showing the withering trichogyne, condition of the chromatophore, the fusion of the stalk-cells, and food particles in the stalk (CLELAND)	340
1.	Inner surface of the cortex of Bombax malabaricum, when producing Mocharas (PETCH)	412
2.	Longitudinal section of the cortex of Bombax malabaricum, when producing Mocharas. A, wood; B, abnormal cortex; C, normal cortex with lignified fibres; D, galleries of Mocharas; E, browned tissue, showing extension of Mocharas formation; F, lignified vessels (PETCH)	413
1.	Tragopogon orientalis. Transverse sections of the extreme base of the central cylinder of the stem, numbered from below upwards (WORSDELL)	426
2.	Tragopogon. Longitudinal section of the lower part of the central cylinder of the stem, showing the typical origin and course of the medullary strands in the genus (WORSDELL)	426
3.	Tragopogon orientalis. Segment of vascular ring, showing internal-phloem strands (*mps*) (WORSDELL)	426
4.	Tolpis barbata. Vascular ring, showing transitions between some of its constituents and the medullary bundles (*mb*). Rudimentary medullar strands (bundles and phloem-strands) are shown at *ms* (WORSDELL)	426
5.	Lactuca virosa. Segment of vascular ring from lower part of stem; in the pith are scattered bundles and phloem-strands (WORSDELL)	431
6.	L. virosa. Ditto from upper part of stem, showing arc-shaped internal-phloem-strands (WORSDELL)	431
7.	L. Plumieri. Petiole, showing vascular ring (*vr*) and medullary strands (*ms*) (WORSDELL)	431
8.	L. alpina. Petiole, showing vascular ring and medullary strands (*ms*) (WORSDELL)	431
9, 10.	Crepis biennis. Segments of vascular ring of stem, showing medullary strands (*ms*) in the bay formed by the outgoing median leaf-bundle (*mlb*). One of the lateral leaf-bundles at *llb* (WORSDELL)	434
11.	C. biennis. Petiole, showing the vestigial character of the adaxial portion of the vascular ring (*avr*), the central medullary strands (*cms*), and the peripheral medullary strands (*pms*) (WORSDELL)	435
12.	Picris hieracioides. Vascular ring of stem, showing its vestigial outermost constituents (*vvr*) and two peripheral medullary bundles (*mb*) (WORSDELL)	437
13.	P. strigosa. Vascular ring and medullary bundles (*mb*) (WORSDELL)	438
14.	Helminthia echioides. Segment of cylinder of stem, showing vascular ring and the scattered medullary phloem-strands (*mps*) (WORSDELL)	438
15.	Cynara Scolymus. Segment of vascular cylinder and cortex (*c*) of uppermost portion of peduncle, showing the scattered disposition of the bundles (WORSDELL)	440
16.	C. Cardunculus. Petiole, showing scattered disposition of the bundles (WORSDELL)	440
17.	Gundelia Tournefortii. Segment of cylinder of stem, showing scattered medullary phloem-strands (*mps*) (WORSDELL)	442
18.	Senecio clivorum. Petiole, showing extreme scattered disposition of the bundles (WORSDELL)	442
19.	Petasites officinalis. Peduncle of male plant, showing scattered disposition of bundles (WORSDELL)	443

PAGE

FIGURES.

20. P. officinalis. Petiole, showing well-developed medullary system of bundles. Bundles of adaxial portion of vascular ring at *axv* (WORSDELL). 445
21. Rudbeckia laciniata. Vascular cylinder of stem, showing absence of medullary strands (WORSDELL) 445
22. R. maxima. Ditto, showing presence of medullary bundles (*mb*) (WORSDELL). 445
23. R. maxima. Leaf, showing complete absence of medullary bundles (WORSDELL). 445
24. Echinacea (Rudbeckia) purpurea. Vascular cylinder of stem, showing rudimentary central medullary bundles (*mb*) (WORSDELL) . . 445
25. E. (Rudbeckia) purpurea. Petiole, showing medullary bundles (*mb*). The most peripheral of these (*pmb*) are recent lateral derivatives from the arc-bundles to which they are opposed (WORSDELL) . 446
26. Inula Helenium. Segment of vascular ring of stem, showing medullary phloem-strands (*mps*). Leaf-bundles at *lb* (WORSDELL) . . 448
27. I. Helenium. Petiole, showing well-marked scattered disposition of the bundles, and the small inverted bundles (*pms*) on the ventral side of the median bundle of the arc (WORSDELL) 448
1–5. Transverse sections of leaf-bundles (AGNES ARBER) 461
6. Carludovica rotundifolius H. Wendl. Transverse section of bundle from near growing point of axis (AGNES ARBER) 463
7. Sciaphila purpurea Benth. Transverse section of a bundle from an axis less than 1 mm. in diameter (AGNES ARBER) . . . 463
1. Rhizoclonium hieroglyphicum, (Kütz.) Stockm. A, transverse section of a filament in the autumn condition; B, external view of part of a similar filament; C and D, transverse sections of a very narrow form; E and F, transverse sections of a much larger form; G, part of a longitudinal section of a similar filament (CARTER) . . 470
2. Chaetomorpha, Kütz. A and B, Ch. gracilis, Kütz. Transverse sections. C, Ch. Linum, (Muell.) Kütz. Longitudinal section (CARTER) . 471
Map of New Zealand and outlying islands (WILLIS) 479
1. Four-leaved sterile plant showing current tuber buried about 18 mm. New tuber formed on short stalk at shallower depth (Oct. 1917) (OSBORN) 486
2. Two-leaved sterile plant with current tuber 4 mm. below ground-level. New tuber formed on long stalk. Sunken about 11 mm. (Oct. 1917) (OSBORN) 486
3. Single-leaved plant with current tuber partially exposed. New tuber as yet scarcely swollen, but sunken to 7 mm. (Sept. 14, 1918) (OSBORN) 486
4. Graph showing variation in depth of current and new tubers on 186 plants collected from (approx.) 300 sq. cm. soil (Oct. 1917) (OSBORN) 488
5. Single-leaved plant with current tuber C.T. and two old season's coats, O.T.$_1$ and O.T.$_2$. This plant is entering on the fourth season of growth. At this date the root is short and no new tuber visible (June 13, 1918) (OSBORN) 490
6. Two-leaved plant with current tuber C.T. at 11 mm. depth, new tuber N.T. just forming. Coats of three previous seasons' tubers (O.T.$_1$, O.T.$_2$, O.T.$_3$) are just below ground-level, showing depth adjustment in 1917 (July 11, 1918) (OSBORN). 490
7. Three-leaved plant with current tuber C.T. at 9 mm. depth. Old tuber coats (O.T., &c.) of four previous seasons at shallower depth (July 11, 1918) (OSBORN) 490
8–14. Regenerating leaves collected in field (Oct. 1917). 8. Detached leaf lying horizontally on soil showing regeneration. A stalk's minute

PAGE

FIGURES.

tuber has formed from the cell mass developed at proximal end. The scar (*sc.*) is almost obliterated. 9 A. Leaf as collected. 9 B. Same after seven weeks on moist soil. 11 A. Leaf as collected. 11 B. Same after seven weeks on moist soil. 12 A. Leaf with unusually large cell mass formed at ground-level, also stalk with growing-point beginning to elongate. 12 B and C. Same viewed from proximal end. 13. Single-leaved plant forming adventitious cell mass on leaf above injury, also new tuber; current tuber not shown. Fig. 14. Three-leaved plant with cell mass forming from proximal end of broken leaf (OSBORN) 492

15. Three-leaved plant grown in laboratory. One leaf, injured July 17, 1918, has developed an adventitious tuber (OSBORN) 494

16–22. Leaves of Series B, detached and put on soil July 11, 1918. The dates below in brackets are those on which the figures were drawn. 16 A. Cell mass forming (Sept. 16). 16 B. Same with adventitious tuber (Nov. 16). 17 A. Growing-point just visible on cell mass (Sept. 16). 17 B. Same, showing elongation of stalk (Sept. 24). 18 A. Cell mass with rhizoids and short stalk bearing growing-point (Sept. 16). 18 B. Same developing two growing-points from cell mass, also 'leaflet' (Sept. 26). 18 C. 'Leaflet' enlarged, two tubers forming (Oct. 8). 18 D. Same: tubers are smaller and less regular than those formed singly by other leaves of the same series. Rhizoids absent (Nov. 16). 19 A. Small cell mass, stalk, and growing-point (Sept. 16). 19 B. Same (Nov. 16). 20 A. Portion of leaf showing cell mass. Leaflet stalk and tubers beginning to swell. No rhizoids at this date (Oct. 8). 20 B. Same original leaf beginning to rot (Nov. 16). 21 and 22. Two remaining leaves of series (Nov. 16) (OSBORN) 496

23–29. Leaves of various series. The dates below in brackets are those on which the various figures were drawn. 23. Leaf of Series C. The cell mass is nodulose; a transverse section of this leaf is seen Fig. 38 (Sept. 24). 24 A and B. Leaf of Series C. This leaf curved on soil in horizontal plane. Adventitious growth from convex side (Nov. 16). 25. Leaf of Series D. Note absence of definite growing-point and formation of many starchy cell masses (Oct. 21). 26–29. Leaves of Series E (Nov. 25) (OSBORN) 499

30–35. Leaves of Series A, detached and put on soil June 13, 1918. The dates below in brackets are those on which the figures were drawn. 30. Adventitious tuber arising from side (Nov. 16). 31. Extreme case of leaf curvature in vertical plane. Cell mass nodular (Sept. 16). 32 A. Leaf showing vertical curvature. This leaf appears to be an exception to rule that adventitious growth occurs from convex surface (Sept. 16). 32 B. Same, viewed from above; note nodular development of cell mass. This leaf was laid flat on soil Sept. 16 (Oct. 21). 32 C. Same; note many short-stalked, white, swollen bodies (Nov. 27). 33 A. Leaf viewed from above, and B, viewed from side, showing geotropic curvature of the many minute, stalked tubers (Nov. 27). 34 A. Leaf viewed from above, and B, viewed from below (Nov. 26). 35. Portion of leaf, viewed from side, with several scattered centres of adventitious growth. Two have developed leaflets and small tubers (Nov. 17) (OSBORN) 502

36. Portion of transverse section of leaf showing adventitious growth from single epidermal cell. Stomate seen to left (OSBORN) 503

37. Transverse section of leaf showing considerable cell mass of epidermal origin, interrupted by stomate at *St.* Growth is becoming localized by development of growing-point seen at right (OSBORN) . 503

PAGE

FIGURES.

38. Transverse section of leaf (see Fig. 23). Two growing-points are developing; the anticlinal walls separating the groups of epidermal cells involved are plainly seen (OSBORN) 503
39. Portion of longitudinal section showing junction of leaf tissue and cell mass showing epidermal origin of latter. Cell mass developed stalk in which a 'tracheide' is seen (OSBORN) 503
40. Transverse section of leaf; epidermis indicated by faint line. An irregular cell mass has formed, and the growing-point has begun to invaginate (OSBORN) 505
41. Portion of longitudinal section through cell mass, stalk (which was accidentally bent), and tuber. 'Vascular supply' seen from its expansion in cell mass to cup of 'tracheides' around growing apex (*x*). Apex projecting into the chamber and seated on spherical mass of starchy tissue. The whole length of channel shown (OSBORN) . 505
42. Longitudinal section of adventitious growth on leaf in Fig. 20 B. Apex (*x*) seen projecting into chamber; extent of starchy tissue shown by faint line. Greater part of 'vascular supply' seen extending towards 'leaflet' (*l*) (which passes out of plane of section), but no 'leaflet' bundle differentiated (OSBORN) 505
43. Detail of apex of tuber seen Fig. 41. Starchy tissue indicated by heavier outline to cells (OSBORN) 506
1. This figure is redrawn from Matthaei's Fig. 3, rectifying a mistake and adding, from her tables, two omissions (SMITH) 522
2. Reinke's results. Curve A. Average of all results in Tables I to VIII. B. Average of all results except those obtained after the plant had been once exposed to 16 units of light (SMITH) 533

William Gilson Farlow.

THE news of Professor Farlow's death on June 3 has been received with sincere regret by British botanists, more especially by those who are connected with the 'Annals of Botany'. All who have had the pleasure of working with him mourn the loss of a loyal and able colleague and a genial friend.

It is only fitting that some notice of him should appear in this periodical, of which he was formerly one of the editors: but nothing like an exhaustive account of his life or a critical estimate of his writings need be attempted. It will suffice to give, in this short sketch, some general appreciation of his significance as a botanist.

William Gilson Farlow was born in Boston on December 17, 1844. He studied at Harvard, and concluded his University career by taking the degree of M.D. in 1870. Although he graduated in Medicine, his intention was to devote himself to Botany, no doubt inspired by Asa Gray. How he proceeded to carry out this intention is told in a pamphlet, 'Cryptogamic Botany at Harvard University, 1874–96', published when his life-work was about half done. He narrates how he was invited by Asa Gray in 1870 to give instruction in Cryptogamic Botany, and how impossible he found it to acquire in America even a passable knowledge of the subject that he had to teach. Consequently he came to Europe for instruction, and spent two years studying in Germany and France.

It may be remarked incidentally that a considerable portion of this time was spent in the laboratory of de Bary at Strasburg. Whilst working there, he made the interesting discovery, which has made his name familiar to all botanists, that the prothallus of certain Ferns gives rise vegetatively to young Fern-plants (Bot. Zeitg., 1874). This remarkable substitution of vegetative propagation for sexual reproduction was subsequently further investigated by de Bary and termed 'apogamy' (Bot. Zeitg., 1878). Farlow's attention was, however, mainly directed to Algae and Fungi, a study in which at that time de Bary was pre-eminent.

On his return, fully equipped, to America, Farlow was appointed Assistant Professor of Botany at Harvard (1874). In the pamphlet he tells by what slow and painful degrees he established a laboratory and accumulated a herbarium for the proper teaching and study of his subject. His efforts were crowned with such success that they were soon recognized by his promotion to full professorial rank as Professor of Cryptogamic Botany

[Annals of Botany, Vol. XXXIII. No. CXXXII. October, 1919.]

(1879), a title which he bore until his death although he had not been engaged in active teaching for some years previously.

The pamphlet contains striking evidence of the activity that prevailed in the cryptogamic department during the first twenty years of its existence, in the form of a list of the numerous papers, all relating to Algae or Fungi, published as the outcome of work accomplished within it. Among the authors, besides Farlow himself, are many whose names have since become well known, such as Thaxter, Humphrey, Davis, Richards, Burt, Peirce, Galloway. Were there a similar record of the doings of the department during the succeeding twenty years, it would be at least as brilliant as that of the earlier period.

Farlow goes on to contrast the position of his study at the beginning and at the end of the period under review. He points out that whereas in 1872 he had 'found it impossible to obtain instruction in Cryptogamic Botany anywhere in the United States, there are in 1896 many institutions scattered over the country where a student can in a few weeks acquire the knowledge which it took the writer several years to gather together in different European countries'. It is for us to say, what Farlow himself could not say, that this great change was, directly or indirectly, due to his own efforts. When it is remembered that these 'institutions scattered over the country' taught not only Cryptogamic Botany but all the other branches of the science as well, some idea can be formed of the revolution that had been brought about, affecting the national attitude towards Botany so profoundly that in no country in the world has botanical organization, both academic and practical, become more extensive and efficient than in the United States. Farlow had brought back with him from Europe not only the information necessary for his work, but also, what was far more important, the inspiration of the modern spirit, of which, as he points out, Sachs's 'Lehrbuch' was to him the embodiment. Without at all under-estimating the value of his published work, it may be truly said that Farlow's real significance is that of a pioneer or missionary.

He readily associated himself with those who had been working on similar lines in this country, when they founded a modern and adequate periodical for the publication of the results of botanical research. He at once accepted the position of American Editor, and his name appears on the title-page of vols. i–xx of the 'Annals of Botany' (1887–1906). His co-operation was invaluable, and contributed largely to establish the friendly relations existing between English-speaking botanists on opposite sides of the Atlantic.

S. H. V.

On the Fertile Shoots of Mesoxylon and an Allied Genus.

BY

D. H. SCOTT, F.R.S.

With Plates I–III and three Figures in the Text.

CONTENTS.

	PAGE
THE FERTILE SHOOTS OF *MESOXYLON MULTIRAME*	1
DESCRIPTION OF THE SPECIMENS	1
NATURE OF THE 'APPENDAGE'	7
ANATOMICAL DETAILS	7
DISCUSSION	8
OTHER SHOOTS ASSOCIATED WITH SEEDS	12
DISCUSSION	14
STEMS OF THE *MESOXYLON* TYPE WITH A SINGLE LEAF-TRACE—A NEW GENUS	15
DISCUSSION	16
CONCLUSION	17
REFERENCES	19
EXPLANATION OF THE PLATES	20

THE FERTILE SHOOTS OF *MESOXYLON MULTIRAME*.

IN a recent paper on the structure of this species of *Mesoxylon* (Scott, 1918, p. 453) it was pointed out that the axillary shoots are branch-systems of a special kind, entirely different from the relatively main axis which bears them. They are characterized by the bilaterally symmetrical organization of the shoot, with a much flattened stele, and by the distichous branches, which bear reduced leaves or bracts, as well as other appendages which may be either prophylls or secondary branchlets. The shoots are closely associated with seeds (*Mitrospermum compressum*, A. Arber) and may have been the organs which bore them. They are termed 'fertile shoots', as there is little doubt that they were connected with reproduction. In the present communication it is proposed to describe these organs fully, and to discuss the evidence available as to their function.

Description of the Specimens.

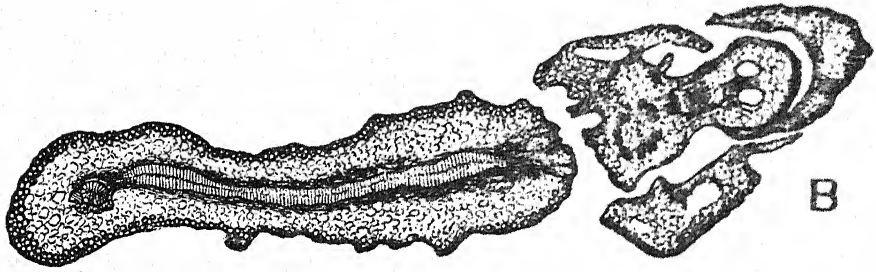
In some ways the most important specimen is one shown in immediate association with a stem of *M. multirame*[1] bearing axillary shoots, for here we have direct evidence for the fertile shoot belonging to the plant. This

[1] Sections Scott Collection 2564–70.

[Annals of Botany, Vol. XXXIII. No. CXXIX. January, 1919.]

specimen will be considered later, but in the first instance it will be most satisfactory to describe a much better preserved example from another series;[1] though not actually associated with a stem, many leaves of the *Mesoxylon* type are present, and the structure of the shoot itself leaves no doubt as to its nature.

The first section of this series is an important one and is fully illustrated (Text-fig. 1; Pl. III, Figs. 17–19). The main part of the specimen is a much flattened, apparently naked axis, in approximately transverse section, measuring about 6·5 × 1·5 mm. (Text-fig. 1). It has an extremely long and narrow stele, just as in the well-known axillary shoots of *M. multirame* (Scott, 1918, Pl. XIII, Fig. 22). The secondary wood is 3–5 elements thick; where the stele is least collapsed, groups of irregularly arranged elements with somewhat thick walls are seen at the inner edge of the



TEXT-FIG. 1. Transverse section of fertile shoot, A, and its branch, B. For details see Plate III, Figs. 17–19. × 12. S. 2781. From a drawing by Mr. G. T. Gwilliam.

secondary zone, and may possibly represent the centripetal xylem of the stele, though the other sections lend little support to this interpretation (Pl. III, Fig. 18). The stele is branching; at one end a small round stele with little or no pith is passing out, and immediately beyond it a small distal bundle is seen (Pl. III, Fig. 19). These features are very constant wherever this phase of branching is observed.

At the opposite end of the section (B) there is a large branch detached, but obviously broken off from the main shoot (Text-fig. 1; Pl. III, Fig. 17). It bears bracts, seen both in connexion with the branch and just free; each bract has a single vascular bundle. The branch itself has a rounded stele, with its bundles somewhat widely spaced, and a considerable pith.

The branch is also giving off a distal appendage (cut obliquely), clearly subtended by one of the bracts, which ensheathes it (Pl. III, Fig. 17). This appendage therefore seems to be a secondary branchlet. The presence of

[1] Sections S. 2781–95. All the specimens described in this paper are from Shore, Littleborough.

an appendage has been observed in several cases, but its nature is open to question (see p. 7).

The main shoot has a certain amount of hypodermal sclerenchyma which extends into the branch, and is especially well developed on the abaxial side of the bracts.

The next section (2782) is not so complete, but at the end (A) where the little round stele was given off in 2781, we now see the base of a branch attached, with a round medullate stele just like that of the former branch. It thus appears that the relatively large stele of the branch, with its pith and distinct bundles, is an expansion of the little pithless stele first given off from the main stele of the shoot. The relative dimensions are—for the branch-stele when first given off, about 0·25 mm.; for the stele when it has passed into the branch, about 0·7 mm.; measuring to the outside of the wood only in each case. The branch seen in the previous section (at the end marked B) is in this case (2782) only represented by bracts.

In the third section (2783) (Pl. I, Fig. 1) the branch of which we saw the base before (at the A end) is here quite free. The bracts and their bases are well shown, but the stele has perished. At the opposite end of the section (B) another little stele is being given off; it is cut obliquely enough to show that tracheides extend to the centre. There are some irregularities in the wood of the main stele here which may be pathological. Off this end a branch or bud is present in oblique section—perhaps the upper part of the first branch.

In the fourth section (2784) a little branch-stele is again detached (at the A end). It shows the small distal bundle, which here seems to have just separated from the branch-stele.

In the fifth section (2785) the branch-stele (at the A end) has moved farther out; it has expanded slightly and acquired a pith. The little distal bundle has moved far out into the cortex. Beyond the opposite end there are some bracts, cut longitudinally, and at a greater distance are two buds in transverse section, each showing both the bracts and a stout appendage lying outside the bract-cycles (Pl. I, Fig. 2). The presence of these buds is explained by the next sections, which show that the axillary shoot curved, so as to be cut in a more longitudinal direction in the later sections; the buds evidently belong to the part of the shoot which runs almost longitudinally. It is not necessary to follow the series farther in detail; enough has been said to show that the axillary shoot gives off distichous leafy branches alternately, the plane of branching being that of the flattened stele of the shoot. The branch, besides the small leaves or bracts, bears a further appendage, the nature of which is discussed below (p. 7).

Anatomically each branch receives a stele from the main shoot; it is small when first given off, but rapidly expands and becomes medullated. Each bract contains a single vascular bundle, while the appendage has

a minute stele or mesarch bundle. In the later sections of the series another axillary shoot (in 2793) and two or three more branches or buds are met with. In two of these (in slide 2789) there is an appendage in addition to the bracts, like that shown in Pl. I, Fig. 2. In one case it is possible that a second appendage is present in the same bud.

The fertile shoot is associated, though not very closely in this case, with a number of seeds. In the series of fifteen sections twelve distinct specimens of *Mitrospermum compressum*, A. Arber, occur. They present all the characters of the species, and there is no doubt as to their identification (see p. 18). None of them, however, show any special relation to the fertile shoots, so the evidence is simply that of association, and such force as it has depends on the high number of the associated seeds. One of the *Mitrospermum* seeds (shown in sections 2792–4) appears to be young, judging from the comparative thinness of the cell-walls of the sclerotesta, but though this seed is in the neighbourhood of the second axillary shoot there is no indication of any connexion.

Some of the sections of the *Mitrospermum* are interesting in themselves; in one (section 2781) the prothallus is preserved; another (2787) shows four pollen-grains in the pollen-chamber, and a third (2791) passes through the chalaza. Though these details are irrelevant to our immediate question, it has been thought worth while to figure the two former (Pl. II, Figs. 15, 16).

It is fair to mention that other seeds, *Physostoma elegans* and *Conostoma oblongum*, occur in the series, but only a couple of specimens of the former and one of the latter.

We have next to consider the specimen of a fertile shoot associated with a vegetative stem of *M. multirame*.[1] The stem is of the ordinary type, about 2 cm. in diameter, with a pith varying from about 7 to 9 mm. in diameter in different parts of the specimen, and wood from 2 to 2·5 mm. in thickness. It is thus a rather small and young stem (Pl. I, Fig. 4).

All the distinctive characters of the species, especially the very gradual convergence of the twin-bundles of the leaf-trace at the margin of the pith, are shown, though the preservation is not specially good. The stem, particularly in its lower part, bears a number of axillary shoots, of which the characteristic steles are conspicuous (Pl. I, Fig. 4); they have the usual tangentially elongated or flattened form, and their secondary wood is thicker on the inner than on the outer face.

The fertile shoot lies quite close to the stem (Pl. I, Fig. 4); it is not much flattened, though the outline is irregular and distorted. The transverse dimensions are roughly 2·9 × 2 mm. In the section in which the shoot first appears (2564) the main stele is badly preserved, but at one end (A) a small, round, pithless stele has been given off, and there are traces of a minute distal

[1] The vegetative stem runs through the series 2563 to 2574, from below upwards; slides 2984 and 2985 appear to be of the same stem, as are also the longitudinal sections 2575–8.

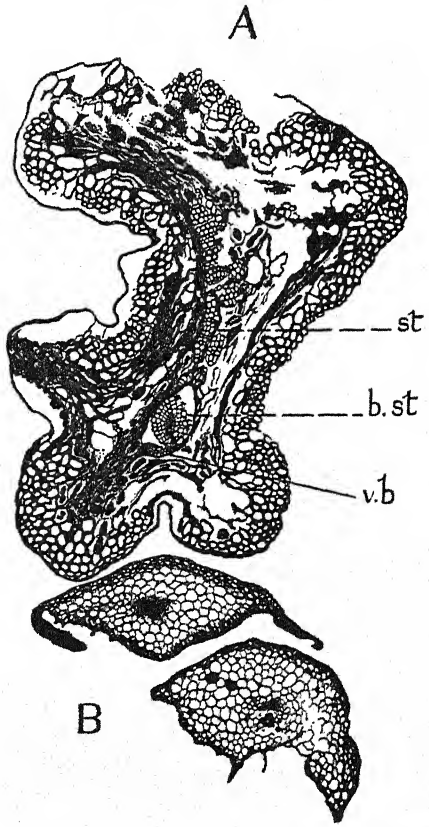
strand. The hypodermal sclerenchyma of the shoot is strongly developed. At the opposite end (B) the shoot bears a large hastate branch, not much smaller than itself, measuring, in the oblique section, about 2·4 × 2 mm. in diameter (Pl. I, Fig. 4, *b*). The three projections appear to be the bases of bracts; no other appendage is distinguishable. The middle is occupied by a large medullate stele, with bundles well separated, just as in the former specimen. The pith has almost perished. The distal arc of the vascular ring projects outwards, but this may be due to accidental causes.

In the next section (2565) the little round stele (at the A end) has enlarged and acquired a small pith; at the opposite end the branch is detached, but the preservation is bad.

The third section (2566) (Text-fig. 2) is favourable for the axillary shoot, in which the very narrow stele is fairly preserved; at one end (B) the usual round branch-stele, with its distal bundle, is passing out. Branches at either end are represented by bracts (shown at B).

The next section (2567) shows little change; the same round branch-stele and distal bundle are present, as before.

In the fifth section (2568) the shoot has diminished somewhat in size (to about 2·5 × 1·75 mm.); it is badly preserved, but bears a large hastate branch, much like that in the first section, but so cut as to appear attached by a narrower base (Pl. I, Fig. 5). At a distance of only 0·8 mm. from the shoot, at the opposite end to the branch, is a seed of *Mitrospermum compressum*, in transverse section. There is, however, no indication of any connexion with the fertile shoot.



TEXT-FIG. 2. Transverse section of fertile shoot, A, and bracts, B. *st.*, main stele of shoot; *b.st.*, stele of a branch; *v.b.*, distal bundle. At B, two bracts, belonging to a branch of the shoot. × 40. S. 2566. From a drawing by Mr. G. T. Gwilliam.

In the following section (2569) little remains of the shoot. A second seed is present, close to the former; it is a rather young *Mitrospermum*, judging from the comparatively thin-walled sclerotesta; again there is no evidence of connexion with the shoot or its branches.

Nothing more of interest is shown in the series. In another section, apparently from the same specimen (2985), a detached axillary shoot is present close to the stem, but it does not show branching, for it is no doubt cut near the base.

I have found no other specimens of *Mitrospermum* in this series besides the two mentioned above. In this case, therefore, their significance depends, not on their number, but on their close association with the fertile shoot. Whether the two seeds were originally attached to the shoot cannot be determined, but it is not improbable that this was the case.

The specimen is inferior to that in the previous series; in particular the organ which we have called the appendage of the branch is nowhere clearly shown, though it was almost certainly present, as the other characters agree so closely with those of the former specimen. The special interest of the shoot just described lies in its close association, on the one hand, with *Mitrospermum compressum*, and on the other with a stem of *Mesoxylon multirame* bearing axillary shoots. From the structure of the fertile shoot, especially its narrow, flattened stele, there can be no doubt that it is of the same nature as the axillary shoots borne on the stem; it evidently constitutes the upper part of a shoot, of which the base may have been still attached to the main stem.

There is another example of a fertile branch shown in a single section (3040); the specimen is associated with leaves of the *Mesoxylon* type, but not with a stem or with seeds. It is the best specimen for the general habit, as it is so little distorted. The shoot is seen in somewhat oblique transverse section, and measures about 5 × 2 mm. (Pl. I, Fig. 6). The identity with the axillary shoots of *Mesoxylon multirame* is manifest (cf. Scott, 1918, Pl. XIII, Fig. 22). The constriction near one end of the section seems to be accidental. The stele is about 2 mm. long by only 0·37 mm. broad, and is thus a good deal flattened, though the whole shoot is not. As pointed out in a previous paper (Scott, 1918, p. 443), the narrow form of the stele in these shoots is no doubt in the main the natural one. The secondary wood is three or four elements thick; no centripetal xylem is preserved. The *Dictyoxylon* outer cortex is well developed; dark elements (secretory sacs?) are, as usual, present in the inner cortex.

At one end of the section the shoot bears a large branch or bud, nearly 3 mm. in diameter (Pl. I, Fig. 6, *b*). It consists of an axis, continuous with that of the shoot, and a number of bracts, several of which are in connexion with the axis, while one is free. The large medullate stele of the branch (0·8 mm. in diameter) is cut obliquely. On its distal side several leaf-trace bundles are seen on their way out to bracts. The dorsal bands of fibres in the bracts are well shown.

The specimen thus exhibits the general characters of the fertile shoot clearly, but affords no new data. No distal appendage is shown, but the plane of section would probably have missed it.

Nature of the 'Appendage'.

The nature of the appendage of the branch seen in several sections of the first series described (from 2781 onwards) remains doubtful. From the evidence of the section represented in Text-fig. 1 and Pl. III, Fig. 17, it would appear that the appendage is a secondary branchlet, for it is clearly subtended by a bract. The other four cases observed (a typical example is shown in Pl. I, Fig. 2) are all from detached buds or branches. In all of these the appendage lies outside the regular cycles of bracts, and is of relatively large size; in the case figured, for example, it measures quite 1 mm. in diameter, while all the rest of the bud is only about 1·7 × 1·3 mm. The appendage is not subtended by a bract in any of these examples, though in that figured there appear to be one or two on one side of it. The structure of the appendage does not suggest a branchlet; it rather resembles a somewhat large bract cut through its basal part; it is flattened or grooved towards the axis of the branch. The slender vascular strand in the case figured has a square xylem-group with the smallest elements almost central (Pl. I, Fig. 3); it thus appears to be mesarch, and does not differ essentially from the bundle of a bract. The distribution of the fibrous tissue is also similar in the appendage and the bracts; in both, at a little distance above the base, it is chiefly concentrated on the abaxial surface, where it forms three or more bands, one being median.

On the whole, therefore, the evidence, apart from the case of the section 2781 (Text-fig. 1), appears to be in favour of the appendage being itself of the nature of a bract. It is possible that the appearance of a branchlet subtended by a bract in the case cited may be deceptive, for here also one might interpret the appendage as a bract, cut very obliquely and still in connexion with the branch, the apparently subtending bract being merely one of an overlapping series.

In the other cases, where the appendage lies to the exterior of the bract-cycles, one might interpret it as the prophyll of the branch, probably supplied by the little bundle given off from the branch-stele near its base (see Pl. III, Fig. 19 and Text-fig. 2). In any case it cannot be said that there is any sufficient evidence for the appendage representing the pedicel of a seed, as at first seemed probable.

Anatomical Details.

A few points in the anatomical structure of the fertile shoot and its branches remain to be considered.

The wood generally has been found to consist of spiral and scalariform elements, thus resembling the inner zone of the wood in the vegetative stem. Considering the thinness of the wood in the shoot and branch, this is not surprising.

As already mentioned, there is in one case (Pl. III, Fig. 18) some evidence for the presence of centripetal xylem in the stele of the main shoot. There is, however, no indication of the protoxylem between the centrifugal and the apparently centripetal elements; neither do the other sections confirm the presence of the latter. Most probably such appearances are merely due to displacement and compression of portions of the centrifugal wood. One would not, in fact, expect centripetal xylem to be represented in the main shoot; in the vegetative stem it is always associated with the leaf-traces, and, as the axillary shoot is leafless, there are no leaf-traces here.

In the branch the case is different, for here the stele receives the traces of the numerous bracts, so that we may expect to find centripetal xylem. But, unfortunately, the preservation is never good enough to show the structure clearly. In the best section for the branch-stele (2781) (Pl. III, Fig. 17) one can see that the greater part of the xylem of the bundles is centrifugal and in radial series; a few irregularly arranged elements on the inner margin may probably represent the centripetal part of the xylem. This applies to the branch-stele after it has expanded and acquired a pith; where it is first given off from the main stele it has a purely centric structure, with neither centripetal xylem nor pith (Pl. III, Fig. 19 and Text-fig. 2).

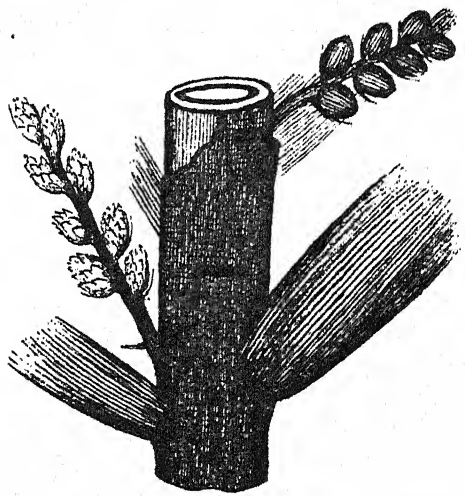
There is no doubt that the bundles of the bracts themselves are mesarch, with a fair amount of centripetal xylem. The case figured with the protoxylem nearly central (Pl. I, Fig. 3) is from an 'appendage', but, as explained above (p. 7), the structure does not differ from that of an ordinary bract.

The distribution of the sclerenchyma in the axillary shoot is in the usual form of a *Dictyoxylon* hypoderma, sometimes nearly continuous (Text-figs. 1 and 2; Pl. I, Figs. 1, 5, and 6). The branch has little free surface, but on the bracts themselves the fibrous tissue is often well developed, occurring on both surfaces but chiefly on the distal side, where it forms several often more or less confluent bands (Text-fig. 2, Pl. I; Figs. 2 and 6; Pl. III, Fig. 17). The parenchyma of the cortex contains sacs with dark contents, similar to those occurring in the vegetative stem (Scott, 1918, p. 452).

Discussion.

The very peculiar characters of the shoots under consideration, and in particular their bilateral symmetry and distichous branching, at once distinguish them from the vegetative axis and indicate a special function. As Grand'Eury said, in speaking of one of his species of *Cordaianthus*: 'La disposition distique des bourgeons est le signe d'une nouvelle destination' (Grand'Eury, 1877, p. 229). On general grounds there could be no

reasonable doubt that the function of such highly modified shoots was connected with reproduction; as a matter of fact there are the closest analogies with various well-known Cordaitean fructifications, and *Mesoxylon*, as we know, was a near ally of *Cordaites*. Grand'Eury says of *Cordaianthus*, 'Les bourgeons floraux plus ou moins nombreux ont une disposition généralément distique' (l. c., p. 227). Our axillary shoot of course corresponds to the main axis of the inflorescence, which Grand'Eury describes as 'fleshy', and the distichously arranged branches to the floral buds. Some of Grand'Eury's figures of *Cordaianthus* agree remarkably well with our specimens, and might almost serve as restorations of them. Attention may be especially directed to *Cordaianthus baccifer* (l. c., Pl. XXVI, Figs. 10, 12, 13) and to the illustrations in Pl. XXV, which show the inflorescences as borne on the stem; one of them is reproduced in our Text-fig. 3. The 'naked peduncle' (e. g. *C. glomeratus*, l.c., p. 230; cf. *C. gemmifer*, Pl. XXVI, Fig. 5) is clearly comparable to the leafless main axis of our axillary shoot. Grand'Eury lays stress on the absence of bracts, flowers, and leaves on the peduncle (l. c., p. 228).



TEXT-FIG. 3. Part of a branch of *Cordaites laevis* (restored). It bears several leaves and two inflorescences, the upper ♀, the lower ♂, with distichous floral buds. From Grand'Eury. About natural size.

The dimensions of the smaller specimens of *Cordaianthus* are quite comparable to those of our specimens. It may be further pointed out that in Grand'Eury's figures showing the inflorescences *in situ* (l.c., Pl. XXV) their insertion lies some little distance above the subtending leaf. This agrees with the position of our axillary shoots (Scott, 1918, p. 448). In position, form, size, and general morphology our fertile shoots thus agree with the inflorescences known as *Cordaianthus*. One point of difference may be mentioned. Grand'Eury (l.c., p. 228) describes the floral buds as borne in the axils of bracts, which are shown in many of his figures, though they are sometimes abortive. There appears to be no subtending bract to the bud or branch in our specimens, for the distal bundle, which might have been interpreted as the trace of such a bract, springs from the branch-stele, and not from the stele of the main shoot (see Pl. III, Fig. 19, and Text-fig. 2). It would thus seem to have supplied a prophyll of the branch, rather than

a subtending bract. The distinction between the two would, however, be scarcely recognizable in specimens preserved as impressions, and the difference is of little significance.

We now come to the comparison of the actual floral buds or branches with those of *Cordaianthus*, and here there is more opportunity for detailed correlation, for fairly full data are supplied by the work of Renault and especially by the later investigations of Bertrand, while, so far as I know, no previous observer has described the structure of the main axis of the inflorescence. Renault (1879, Pl. XVII, Fig. 1) shows a transverse section of the axis of *Cordaianthus subglomeratus*, with some indication of the vascular ring, but here there is little analogy with our specimens, as the floral buds are not distichously arranged.

Renault (1879) has little to say about the structure of the axis and bracts of the floral buds; his attention was concentrated on the stamens and ovules. His figures, however, show the bracts arranged spirally in numerous cycles, each bract having a single vascular strand (l. c., Pl. XVI, Figs. 12–15; Pl. XVII, Figs. 1–3, 11, 13, 14). The bracts of the female catkin are described as thicker and more coriaceous than those of the male (l.c., p. 312).

Much fuller details are given by the late Professor C. E. Bertrand in his paper on the Female Bud of *Cordaites* (Bertrand, 1911). His observations are confined to the detached floral bud or catkin (which he sometimes calls the 'inflorescence'), and there is no reference to the main axis, which appears not to be represented among Renault's preparations on which the account is based.

The axis of the young bud, he says, has no free surface; it is covered by the bases of the bracts (l. c., p. 25). The vascular ring consists of about ten isolated strands surrounding a pith. Each strand includes on the inner side an irregular group of spiral elements, with 2–4 groups of radially arranged secondary elements on the exterior. The illustration (l. c., Pl. V, Fig. 37) shows that the structure of the stele of the bud essentially agrees with that in our specimens (cf. Pl. III, Fig. 17). A detailed comparison of the other tissues is superfluous, as the preservation is poor both in his material and ours.

The bracts in Renault's specimens are remarkably like ours. Those figured by Bertrand (l. c., Pl. V, Fig. 43) are almost identical with the bracts of the bud shown in Pl. I, Fig. 2 (see also Text-fig. 2); the form of those cut near the base is the same, and that of the more distal bracts very nearly so. The bracts, however, are much less numerous in our specimens, perhaps because some are lost. The structure of the bracts in the French specimens is described by Bertrand in great detail (l. c., pp. 30–7). Here it may suffice to mention that in the free, middle part, the bract has two bands of fibres on the distal face, and a less developed fibrous layer

on the inner side. In ours the distal bands, where they are distinct, are more numerous. The parenchyma is large-celled, especially towards the middle, just as in ours. The vascular bundle is described as identical with a single nerve of the vegetative leaf of *Cordaites* (l. c., p. 34); in our specimens it is mesarch, which comes to the same thing. Bertrand's Fig. 41 shows a bundle exactly like that of an imperfectly preserved bract in one of our specimens. In the basal part of the bract the fibrous bands disappear just as in ours. Bertrand speaks of hairs on the bracts, which have not been detected in our specimens.

Considering that there is no question of specific identity, the agreement between the branches in our specimens and the floral buds of the French fructifications is remarkably close. Taking both general morphology and detailed structure into consideration, we may conclude with confidence that our fertile shoots with their branches are of the same nature as the inflorescences of *Cordaites*; they constitute, in fact, the *Cordaianthus* of *Mesoxylon multirame*.

The question remains, Of what sex was this *Cordaianthus*? The detailed comparison has been with a female fructification, but there was little difference in general morphology and structure between the two sexes. Male catkins, however, are short-lived organs, and it is hardly likely that we should find the accessory parts mature and fairly preserved without some trace of the stamens themselves. Also any force that association may have tells in favour of the fertile shoots having been seed-bearing organs. Supposing that this was their nature, it is probable that some of the Mitrospermums which we find in the neighbourhood of the fertile shoots, especially the younger seeds, may have been borne on them and become detached. In the best known female specimens of *Cordaianthus* the seeds are borne laterally on the catkin, each terminating a short pedicel, probably in the axil of a bract (Bertrand, 1911, p. 37). The ovules are surrounded by the bracts of the catkin, from among which the ripening seed may emerge. We have found no satisfactory evidence of the presence of seed-pedicels, for the 'appendage' appears to have been of the nature of a bract, except perhaps in the one case shown in Text-fig. 1 and Pl. III, Fig. 17, and even here, as we have seen, the interpretation of the appendage as a branchlet or pedicel is open to some doubt. Unfortunately, then, we are at present unable to explain how the seeds were borne, and the solution of the question must await the discovery of more perfect specimens. Possibly the seed may have been terminal on the branch.

In any case the nature of the fertile shoot of *Mesoxylon multirame* as a *Cordaianthus* has been sufficiently demonstrated.

Other Shoots associated with Seeds.

We have now to consider two examples of shoots which show in their structure no relation to *Cordaianthus*, and indeed resemble vegetative buds rather than inflorescences, but are more or less closely associated with seeds of the *Mitrospermum* type. As we shall see, they show an unmistakable relation to *Mesoxylon*, though there are reasons for doubting whether they can properly be included in that genus.

The specimen to be first described is represented by seven transverse sections, the series running from below upwards.[1] The axis, with the leaf-bases, has a diameter of about 8 mm. The whole surface is clothed with the massive leaf-bases, the true cortex being comparatively narrow (Pl. I, Figs. 7, 8). At some places the leaf-base is seen expanding into the lamina, and imperfectly preserved laminae of other leaves surround the specimen.

There is a somewhat narrow zone of wood, about 10–20 elements in thickness; in places the centripetal xylem of the leaf-traces can be recognized, most clearly in the second section (2599). The most interesting point is that the leaf-traces are single where they pass through the wood. They run out horizontally, so that they can be traced far out into the leaf-base, forking once or twice after reaching the cortex. Farther out in their course they become more vertical, and undergo repeated divisions, for in the outer part of a leaf-base as many as nine little bundles, cut very obliquely, have been observed (Pl. I, Fig. 7, *l.b.*). The structure of the wood and also the *Dictyoxylon* cortex are like those of a *Mesoxylon*, and the pith, so far as can be judged from transverse sections, appears to have been discoid, for the displaced diaphragms are shown (Pl. I, Figs. 7, 8). Periderm was formed, cutting off the leaf-bases.

The leaves in connexion with or surrounding the axis have many bundles, and are of the ordinary *Cordaites* or *Mesoxylon* type. The xylem of the foliar bundles appears to be mainly centripetal. In the upper sections (e.g. 2603) periderm had formed in the leaves, usually on both surfaces—an interesting point, which strongly suggests a resting, vegetative bud. In the upper part of the specimen the wood diminishes in thickness.

There is nothing in the structure of this specimen to indicate that it was a fertile shoot. It is, however, associated with seeds, apparently of *Mitrospermum compressum*, of which five specimens occur in the series, two of them lying near the bud. One of the seeds shows signs of youth.

[1] The numbers of the sections in my collection are 2598 to 2604. They were received in August, 1910, from Mr. Lomax, who described the specimen as 'a small apical shoot', and called attention to the association with the seeds of *Cardiocarpon* (now *Mitrospermum*) *compressum*.

The other specimen, represented by six sections, is more remarkable, for the axis increases rapidly in diameter from below upwards, and appears to approach the growing point at the upper end.[1] At the base (section 3017) the shoot measures altogether about 4·5 mm. in diameter; the stele is about 1·7 mm., with a minute pith, only about 0·4 mm. in diameter; the wood is of unequal thickness, from 0·37 to 0·9 mm. (Pl. I, Fig. 9). Two single leaf-traces are passing out. The cortex and leaf-bases are obscure in this section, but the axis is surrounded by broad, ill-preserved leaves. In the next section (3018) the diameter of the shoot has increased to 7–8 mm. The stele is incomplete, but evidently larger than before, with the wood 0·53 mm. thick in the part preserved; numerous bundles are seen entering a leaf-base, as in the previous specimen. The third section (3019) (Pl. II, Fig. 10) is a much better one and shows a marked change. The diameter of the whole shoot is here about 8 × 10 mm. The stele measures 3 × 2·5 mm. and the pith, which is pentagonal in form with gaps at the angles, about 1·5 mm. The wood reaches 0·57 mm. (24–30 elements) in thickness. A large single leaf-trace is passing out. The pith here, as in all the sections which show it, seems continuous; there is nowhere any indication of a discoid structure. Centripetal xylem is distinctly shown at certain points around the pith.

In the fourth section (3020) the dimensions have further increased, the whole diameter being about 10 × 15 mm. The stele has a diameter of 3 × 2·8 mm. and the pentagonal pith of 1·8 mm.; the wood has a fairly uniform thickness of 0·4 mm. There is a forking leaf-trace in the cortex, opposite one of the gaps in the ring of wood.

In the two sections last mentioned the large leaf-bases and the encircling leaves are better preserved, though still imperfect; they seem to agree essentially with those of the former specimen; certainly the leaf-bases are relatively more strongly developed than on the vegetative stems of *Mesoxylon*, and the leaves themselves are polydesmic and probably of the Cordaitean type.

In the next section of the series (3021) the axis is destroyed, but the uppermost section (3022) is interesting, for it shows the stele in a very young condition. The preservation is bad on the whole, but a ring of quite isolated, vascular bundles is shown. There appear to be nine of these bundles, though some are obscure, surrounding a large pith and separated by broad principal rays. The secondary wood of each bundle is only four or five cells thick; in some cases a few centripetal elements can be recognized. At one place a leaf-trace is passing out, almost horizontally.

[1] The series is 3017–22, from below upwards. The sections were received from Mr. Lomax in October, 1910. He rightly regarded this specimen also as an apical shoot (possibly, as he then thought, of *Mesoxylon multirame*) and attached great importance to its intimate association with the seeds.

The diameter of the vascular ring is about 2·5 mm.; allowing for the small development of the wood, this is about the full size of the stele in the preceding sections. Evidently we are here approaching the apical region of the bud; it is interesting to find that the vascular bundles are at first widely separated, and that it is only at a later stage that they become united into a more or less continuous vascular zone.

About nine seeds of *Mitrospermum* occur in the series, some of which are evidently young. In section 3019 three of the seeds closely surround the shoot and all appear young, the cell-walls of the sclerotesta being but little thickened. In one of them the indications of youth are especially marked; all the cell-walls are remarkably thin, the megaspore is relatively small, and there appears to be a thick layer of nucellar tissue persisting between the megaspore and the integument.

Discussion.

There can, I think, be no doubt that the two specimens just described are of the same kind. Both are of the nature of buds or apical shoots, as is proved by the leaves wrapped closely round the axis. They agree in the structure of the wood, in the single leaf-traces dividing in the cortex, and in the massive leaf-bases. The chief differences are, first, in the structure of the pith, which seems to have been discoid in the first specimen and continuous in the second. There is a slight difference also in the form of the pith, which is markedly pentagonal, at least where it attains its full size, in the second specimen, and only faintly angular in the first. This, however, is a small point.

Another conspicuous difference is the striking contraction of the whole shoot and its stele towards the base in the second specimen, while the diameter is fairly uniform throughout in the first. This distinction suggests that the second specimen may have been a lateral bud, and the first a terminal one. This suggestion might perhaps also throw some light on the difference in the structure of the pith.

The anatomical habit of both shoots is distinctly that of a *Mesoxylon*; the presence of centripetal wood and the polydesmic structure of the encircling leaves show that the affinity is a real one. But in the important character of the single leaf-trace there is a marked distinction from *Mesoxylon*, for in all the species referred to this genus the trace is double where it passes through the wood. This, in fact, has been made a character of the genus (Scott and Maslen, 1910, p. 237). It is true that in the buds of the fertile shoot of *M. multirame* the leaf-trace is single, but here the reason is obvious, for it supplies a monodesmic bract. In the shoots now under consideration the leaf-base and leaf have many bundles, and yet the trace is single at its origin, only dividing as it passes through the cortex. This peculiar feature might no doubt be due to the shoots being of a special

morphological nature, differing from vegetative branches. They might then belong to some species of *Mesoxylon*. But specimens of ordinary vegetative stems of the *Mesoxylon* type have now come to light which agree with these shoots in having single leaf-traces, and it is suggested that the shoots belonged to a plant of this kind. The stems in question will be described immediately; in the meantime the attribution of the bud-like shoots must be left an open question (see p. 17).

There is nothing to show that these shoots were connected with reproduction; they have all the character of vegetative buds, and bear no resemblance to any form of *Cordaianthus*. If they bore branches of the nature of fructifications, no trace of any such organs is to be found in the specimens. Yet the association with *Mitrospermum* seeds, and especially with young seeds, is striking. Probably it is accidental, for if all the seeds are of the same species they could not have been borne both on these shoots and on the *Cordaianthus* of *Mesoxylon multirame*.

I shall return to the subject of association at the conclusion of the paper.

Stems of the *Mesoxylon* Type with a Single Leaf-trace—a New Genus.

The stems in question are represented only by two isolated transverse sections in my collection.[1] The smaller of the two specimens (2983) is about 11 mm. in diameter in its present condition, but the cortex is incomplete (Pl. II, Fig. 11). The stele (to the outer edge of the wood) measures about 9 × 8 mm. and the pith about 5 × 3·5 mm. The pith is almost destroyed by Stigmarian rootlets, and it is impossible to determine whether it was discoid or not. The small-celled wood is from 1·3 to 2·5 mm. thick. The medullary rays are narrow and uniseriate, and the general structure of the wood, so far as can be judged from a transverse section, is similar to that of a *Mesoxylon*, such as *M. multirame* or *M. poroxyloides*. It also agrees essentially with that of the bud-like shoots described in the preceding paragraphs.

Three leaf-traces, each manifestly a single bundle, are seen on their course through the wood; they pass out obliquely but not horizontally. In two of them the centripetal xylem can be clearly seen, and in one, at any rate, the intermediate position of the protoxylem is evident (Pl. II, Fig. 12). A fourth bundle is just leaving the pith, and this also shows the same mesarch structure. The inner edge of the wood is not well preserved, and though there are indications of centripetal wood at some points, it is nowhere very clear, apart from the outgoing traces. The phloem and

[1] The numbers are 2983 and 2993, both received from Mr. Lomax early in 1916; they were found at Shore, Littleborough.

pericycle are poorly preserved, and the inner cortex, which alone is present, shows a not very distinctive parenchymatous structure.

On the data available it appears that this specimen is a stem allied to *Mesoxylon*; the size, and especially the considerable development of the wood, indicate that it was an ordinary vegetative shoot.

The second specimen (section 2993) is of a much larger stem (Pl. II, Fig. 13). Only the pith and wood are preserved, and the latter is incomplete. The maximum radius, to the outer edge of the wood, is almost 2 cm. The pith is about 6 mm. in diameter; it is nearly complete, but it is quite likely that the plane of section may happen to coincide with a diaphragm. Its contour is obscurely angular. The structure is very uniform, the cells merely becoming smaller towards the outside; many of them have dark contents. The appearance is very similar to that of a diaphragm of *Mesoxylon multirame* when seen in horizontal section.

The structure of the wood is the same as in the former specimen, except that the rays sometimes appear to be biseriate, a feature often met with in species of *Mesoxylon*. It shows a more definite appearance of 'annual rings' than is usual in carboniferous woods; examined with a lens the rings look quite like concentric bands of autumn wood (Pl. II, Fig. 13). But they prove, on closer investigation, not to be really continuous, and though some of the cells are flattened this is not at all generally the case. The distinction appears to depend mainly on a somewhat greater thickness of the cell-walls in these bands.

Two single leaf-traces are seen passing out obliquely through the inner part of the wood (Pl. II, Fig. 13).

At several points of the inner edge of the wood a prominent group of primary xylem is seen, which is evidently centripetal in development, and comparable to the primary xylem of a *Mesoxylon* (Pl. II, Fig. 14). In all cases such groups occur singly, never in pairs; they clearly represent the downward continuation of single leaf-traces which have passed in at a higher level.

Discussion.

These two specimens differ practically in nothing except size, and may safely be referred to the same species. The characters open to investigation are somewhat meagre; in the general structure of the wood and pith (when shown) and in the presence of centripetal xylem, both in the outgoing leaf-traces and in strands at the inner edge of the wood, the stems agree with *Mesoxylon*. They differ from that genus in the important point that the leaf-trace, as it traverses the wood, is single and not double. At the same time it is evident that the specimens are ordinary vegetative stems, and thus directly comparable with those on which the genus *Mesoxylon* was founded. It will be noticed that the pith is smaller than is usual in *Mesoxylon*.

Without altering the generic characters of *Mesoxylon* it is impossible to include the stems in question in that genus, for the generic diagnosis contains the statement: 'Leaf-traces double where they leave the pith, the two strands uniting at a lower level' (Scott and Maslen, 1910, p. 237). There is no question of dropping this character, on which great stress has been laid in all descriptions of the species of *Mesoxylon*. It must, however, be understood that it applies essentially to the vegetative stems only.

It thus becomes necessary to establish a new genus for the specimens just described, and the name I propose to adopt is *Mesoxylopsis*. I think we are logically compelled to include in the new genus the two bud-like shoots (of the 2598 and 3017 series) previously described. They too have single leaf-traces, and the evidence goes to show that the shoots were vegetative organs. The fact that they bore polydesmic leaves shows that the singleness of the leaf-trace was not simply an adaptation to a reduced foliar structure. The case for separation from *Mesoxylon* is in fact just about as strong for these buds as it is for the larger stems just described. Although, therefore, we cannot absolutely prove that the bud-like shoots may not have been peculiar specialized branches of a *Mesoxylon*, the presumption on present evidence is that they belonged to *Mesoxylopsis*, and I have taken account of their characters in drawing up the following diagnosis of the new genus:

MESOXYLOPSIS, gen. nov.

Pith probably discoid in the mature stem.

Wood dense, with narrow, usually uniseriate medullary rays.

Leaf-traces single where they leave the pith and pass through the wood, forking repeatedly in the cortex and leaf-base.

Centripetal xylem present in the leaf-traces at the margin of the pith and throughout their outward course into the leaves.

Leaf-bases massive, crowded.

Leaves polydesmic, of the type of *Cordaites* and *Mesoxylon*.

All the specimens may be referred to a single species which I have pleasure in naming *Mesoxylopsis Arberae*, sp. nov., after Dr. Agnes Arber, F.L.S., who has so kindly aided in the determination of seeds associated with the shoots described in the present paper.[1] The characters of the species are those of the genus. In the specimens observed the pith is relatively small, not exceeding 6 mm. in diameter.

CONCLUSION.

In the first part of the paper the structure of the fertile shoots of *Mesoxylon multirame* is described. They are found to be identical with the axillary shoots, which have been known since the first discovery of the species.

[1] See below, p. 18.

The fertile shoot is bilaterally symmetrical; it consists of a naked main axis, containing a flattened stele, and bearing distichously arranged bud-like branches, lying in the plane of the major axis of the main shoot. Each branch has a cylindrical, medullated stele, and bears numerous spirally arranged bracts, with a single vascular bundle of mesarch structure. A large bract, lying outside the others, may probably be the prophyll of the branch. In one case an appendage, apparently of a different kind, was observed, which might possibly be the pedicel of a seed.

In general morphology, and in the detailed organization of the lateral buds, the fertile shoots show a close agreement with the distichous forms of *Cordaianthus*, described by Grand'Eury, Renault, and Bertrand, and are clearly of the same nature. The sex of the inflorescence has not been determined, as no reproductive organs are attached, but the probability is in favour of its having been a seed-bearing organ. It is associated with the seeds named *Mitrospermum compressum* by Dr. Agnes Arber.

Two shoots of a different kind are then described, which appear to have been of the nature of vegetative buds, for their structure is that of a stem, and they bear closely packed polydesmic leaves, of a Cordaitean type. The leaf-traces of these shoots are single where they traverse the wood, only dividing in the cortex. These shoots also are associated with seeds of *Mitrospermum compressum*.

Lastly, two larger stems of the *Mesoxylon* type are recorded, in which the leaf-traces are likewise single in passing through the wood. These stems and the bud-like shoots are placed together in a new genus, *Mesoxylopsis*, differing essentially from *Mesoxylon* in the leaf-trace being single and not double. The one known species is named *Mesoxylopsis Arberae*.

The equally intimate association of *Mitrospermum compressum* with the *Cordaianthus* of *Mesoxylon multirame* on the one hand, and with the bud-like shoots of *Mesoxylopsis Arberae* on the other, raises grave doubts as to the value of the evidence from association, always so uncertain in palaeobotany, and doubly treacherous among the crowded and intermixed fragments of the coal-ball petrifactions. It was, however, clearly desirable to ascertain whether the seeds of the two categories were in fact specifically identical. Dr. Agnes Arber, F.L.S., to whom I have submitted sections showing the seeds associated with both kinds of shoots, kindly allows me to quote her opinion, as follows: 'The sections of the seeds *all* seem to me to be typical *Mitrospermum compressum*, and I see no reason for separating those in the three slides in which they are associated with *Mesoxylon multirame*.' At the same time Dr. A. Arber calls attention to a remark in her preliminary note on this seed (A. Arber, 1910, p. 393): 'There is sufficient variation among the specimens, both in dimensions and structure, to suggest that *Cardiocarpon* [now *Mitrospermum*] *compressum*, instead of

being a single species, may possibly represent an assemblage of seeds belonging to closely allied plants.'

It is therefore not impossible that seeds of the *Mitrospermum compressum* type, at present indistinguishable from one another, may have been borne both by *Mesoxylon multirame* and *Mesoxylopsis Arberae*. But, in the existing state of our knowledge, we are not justified in making any such assumption, and the significance of the evidence from association in the two cases is at present quite doubtful. Considering, however, that the fertile shoots of *Mesoxylon multirame* have now been shown to agree in morphology and structure with a *Cordaianthus*, it is highly probable that the platyspermic seeds associated with them may have really belonged to the plant.

The genus *Diplotesta*, to which Bertrand referred the ovules of his *Cordaianthus* (Bertrand, 1911), appears to be closely allied to *Mitrospermum* (Brongniart, 1881, Pls. XIII and XIV; Bertrand, 1907), and the latter genus is in every respect a seed of the type which there is good reason for attributing to the Cordaitales (see Seward, 1917, pp. 332–56).

The main result of the present investigation is, however, the proof that *Mesoxylon multirame* bore a *Cordaianthus* in all respects comparable to the inflorescence of *Cordaites*. The close affinity of the two genera and the definite location of *Mesoxylon* in the family Cordaiteae are thus securely established. There is little doubt that the new genus *Mesoxylopsis* is of like affinities, but further evidence is needed before its exact position can be determined.

While the evidence from association with seeds has proved too uncertain to be relied on, great credit is due to Mr. James Lomax for calling attention to the shoots described in the present paper. In the case of the fertile branches of *Mesoxylon multirame* his conviction of their 'fructiferous' nature has been fully confirmed.

The photographic illustrations to the present communication are the work of Mr. W. Tams of Cambridge. The drawings, both the text-figures and those in the plate, are from the pencil of Mr. G. T. Gwilliam, F.R.A.S. To both these gentlemen I desire to return my thanks for their skilful aid.

References.

Arber, Agnes (1910): A Note on *Cardiocarpon compressum*, Will. Proc. Cambridge Philosophical Soc., vol. xv, pt. v, p. 303.

——— (1912): On the Structure of the Palaeozoic Seed *Mitrospermum compressum* (Will.). Ann. Bot., vol. xxiv, p. 491.

Bertrand, C. E. (1907): Les caractéristiques du genre *Diplotesta* de Brongniart. Bull. de la Soc. Bot. de France, 4e série, tome vii, p. 389.

BERTRAND, C. E. (1911): Le Bourgeon femelle des *Cordaites* d'après les préparations de Bernard Renault. Bull. de la Soc. des Sciences de Nancy, p. 1.

BRONGNIART, A. (1881): Recherches sur les Graines fossiles silicifiées. Paris.

GRAND'EURY, C. (1877): Flore carbonifère du Département de la Loire et du Centre de la France. Paris.

MASLEN, A. J. (1911): The Structure of *Mesoxylon Sutcliffii* (Scott). Ann. Bot., vol. xxv, p. 381.

RENAULT, B. (1879): Structure comparée de quelques tiges de la Flore Carbonifère. Nouvelles Archives du Muséum, tome ii, 2e série, p. 215.

SCOTT, D. H. (1912): The Structure of *Mesoxylon Lomaxii* and *M. poroxyloides*. Ann. Bot., vol. xxvi, p. 1011.

——— (1918): The Structure of *Mesoxylon multirame*. Ann. Bot., vol. xxxii, p. 437.

SCOTT, D. H., and MASLEN, A. J. (1910): On *Mesoxylon*, a New Genus of Cordaitales. Preliminary Note. Ann. Bot., vol. xxiv, p. 236.

SEWARD, A. C. (1917): Fossil Plants, vol. iii. Cambridge University Press.

EXPLANATION OF PLATES I–III.

Illustrating Dr. Scott's paper on the Fertile Shoots of *Mesoxylon* and an Allied Genus.

The photographic figures require to be examined with a lens.

PLATE I.

Photographs by Mr. W. Tams.

Figs. 1–6. Fertile shoots of *Mesoxylon multirame*.

Fig. 1. Transverse section of the fertile shoot shown in Text-fig. 1; at A is a detached branch with bracts, and at B the bracts of another branch. × about 7. S. 2783.

Fig. 2. Another detached bud, transverse, with numerous bracts, *br.*, and a large appendage, *ap.* × 22. S. 2785.

Fig. 3. Vascular bundle of the appendage in Fig. 2, showing the central protoxylem, *p.x.* × about 200. S. 2785.

Fig. 4. Part of transverse section of a stem of *M. multirame*, accompanied by a fertile shoot. x^2., secondary wood of the stem; *ax.*, an axillary stele of the same; *F.S.*, main fertile shoot; *b.*, branch attached to it. × about 8. S. 2564.

Fig. 5. Another transverse section of the same fertile shoot; *F.S.*, main shoot; *h.*, large hastate branch attached to it. × 17. S. 2568.

Fig. 6. Another fertile shoot in obliquely transverse section. *F.S.*, main shoot, with narrow stele; *b.*, large branch attached to it, with bracts. × about 10. S. 3040.

Figs. 7–9. Shoots of *Mesoxylopsis Arberae*.

Fig. 7. Shoot in transverse section. *p.*, pith, apparently discoid. $l.t.^1$, single leaf-trace passing through wood; $l.t.^2$, leaf-trace forking in cortex; *l.b.*, leaf-base with numerous bundles; *l.*, leaves surrounding shoot. × about 8. S. 2598.

Fig. 8. Next section of the same shoot. *p.*, pith, as before; *l.t.*, leaf-trace beginning to fork; *l.b.*, leaf-base expanding into lamina on either side; *l.*, leaves surrounding shoot. × about 8. S. 2599.

Fig. 9. Lower part of another shoot in transverse section. *st.*, centre of stele, with small solid pith and very unequal wood in which two single leaf-traces are seen; *l.b.*, one of the leaf-bases. × 17. S. 3017.

PLATE II.

Photographs by Mr. W. Tams.

Fig. 10. From a higher section of the same shoot as Fig. 9, showing the enlarged stele and surrounding tissues. *p.*, the large, solid pith; *l.t.*, a single leaf-trace passing through the wood. × about 15. S. 3019.

Figs. 11–14. Stem of *Mesoxylopsis Arberae.*

Fig. 11. General transverse section. *l.t.*^{1}, single leaf-trace starting from the pith; *l.t.*^{2}, another leaf-trace; *c.*, remains of cortex. × about 7. S. 2983.

Fig. 12. The leaf-trace, *l.t.*^{1}, from Fig. 11 and neighbouring tissues, more magnified. *x.*^{1}, centripetal xylem of leaf-trace. × about 50. S. 2983.

Fig. 13. General transverse section of another stem, showing rings of growth. *p.*, more or less solid pith; *l.t.*^{1}, *l.t.*^{2}, single leaf-traces passing through wood. × about 4. S. 2993.

Fig. 14. The leaf-trace, *l.t.*^{1}, from Fig. 13 and neighbouring tissues more magnified; *x.*^{1}, centripetal xylem of leaf-trace. × about 50. S. 2993.

Figs. 15, 16. *Mitrospermum compressum.*

Fig. 15. Transverse section of seed. *sa.*, sarcotesta; *sc.*, sclerotesta; *pr.*, prothallus, a delicate, imperfectly preserved tissue, filling the megaspore. × about 16. S. 2781.

Fig. 16. Part of obliquely transverse section through upper part of a seed. *sc.*, sclerotesta; *p.c.*, the pollen-chamber, in which four large pollen-grains, *p.g.*, are contained. × 125. S. 2787.

PLATE III.

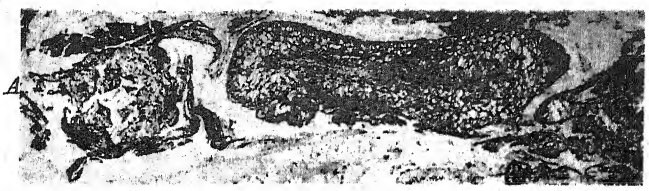
Mesoxylon multirame.

From drawings by Mr. G. T. Gwilliam. All are from the section shown, as a whole, in Text-fig. 1.

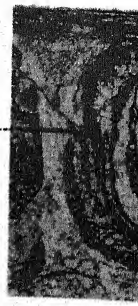
Fig. 17. The branch B of the fertile shoot, in approximately transverse section. *st.*, stele of branch; *br.*, a free bract; *ap.*, appendage; *br.s.*, bract, apparently subtending the appendage. × about 30. S. 2781.

Fig. 18. Part of stele of fertile shoot, transverse. *x.*^{1}, apparent primary wood; *x.*^{2}, secondary wood. × about 170. S. 2781.

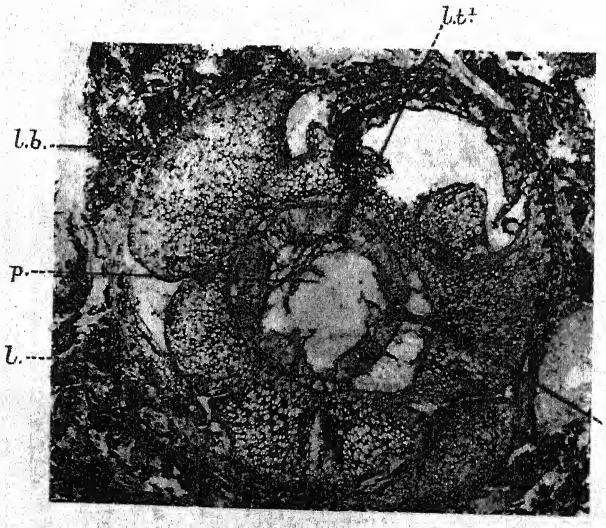
Fig. 19. Part of fertile shoot, at A end, transverse. *st.*, portion of main stele; *st.b.*, stele of a branch; *v.b.*, distal bundle. × about 60. S. 2781.

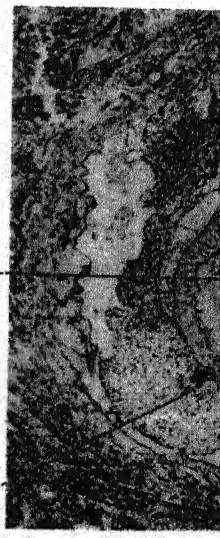


1.



4.

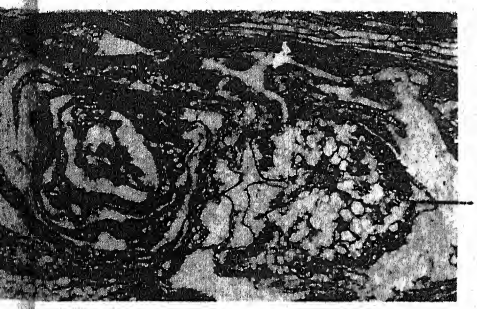




7.

Tams, phot.

SCOTT — MESOXYLON AND MESOXYLOPSIS.



2.

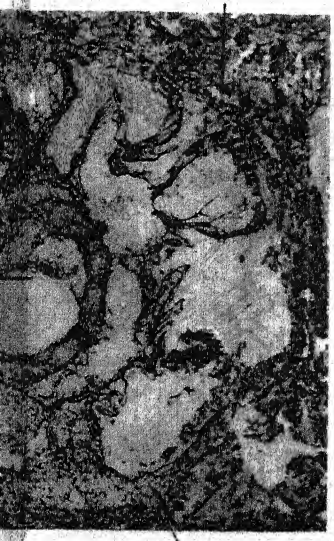
3.

5.

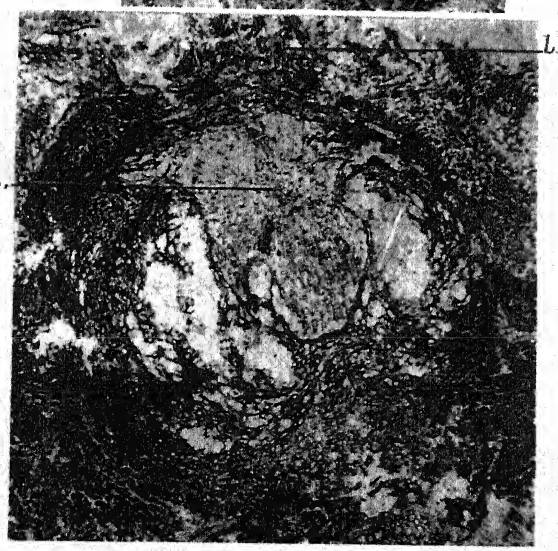
b.

6.

l.

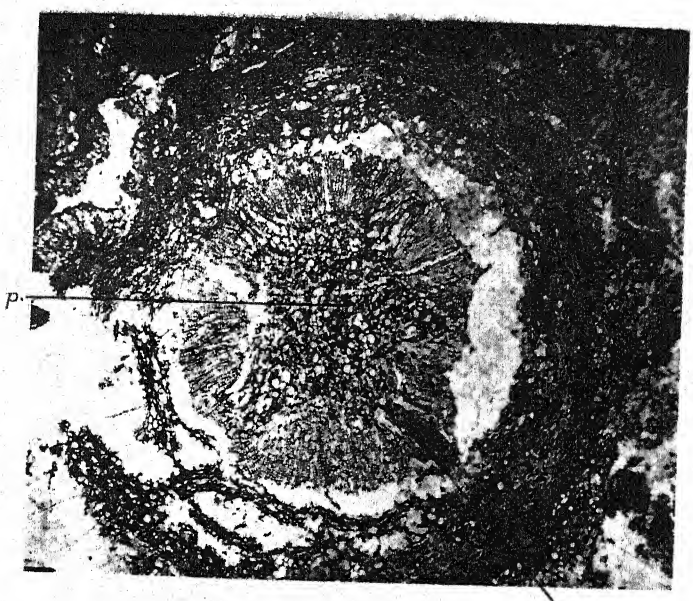


8.

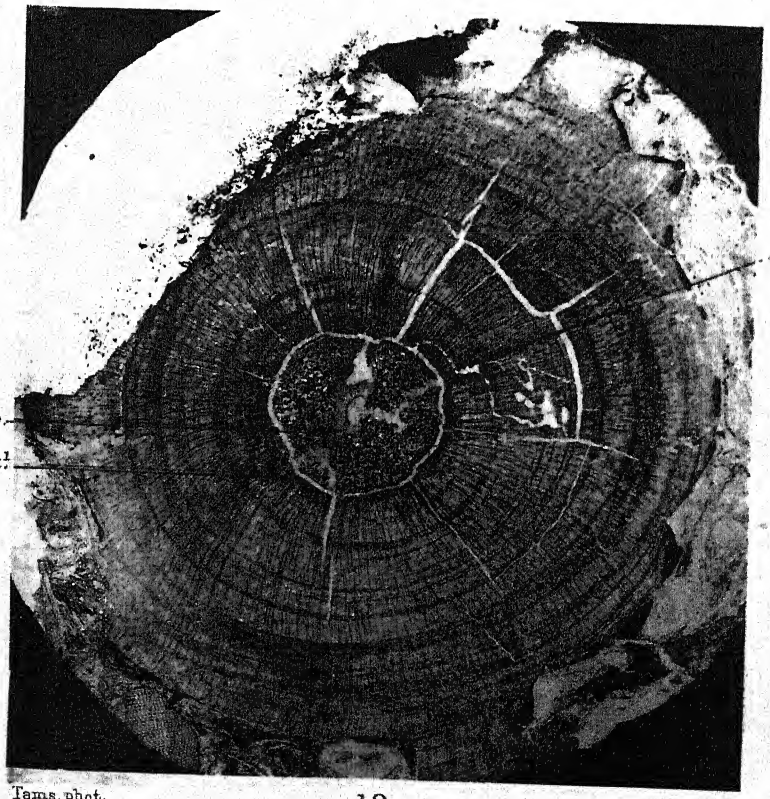


9.

Huth, coll.



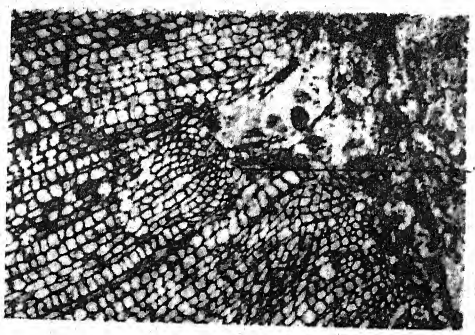
10.



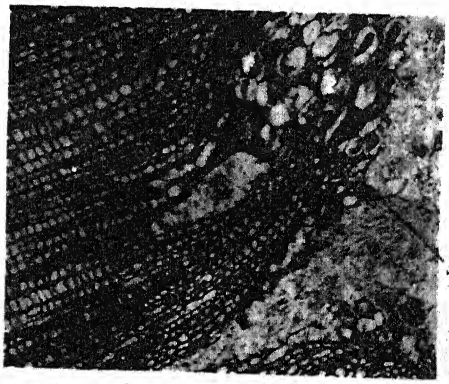
Tams, phot.

13.

11.

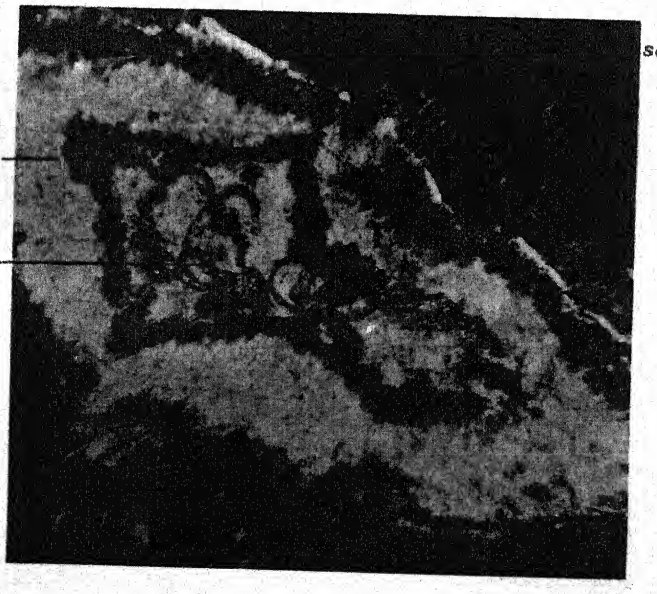


12.



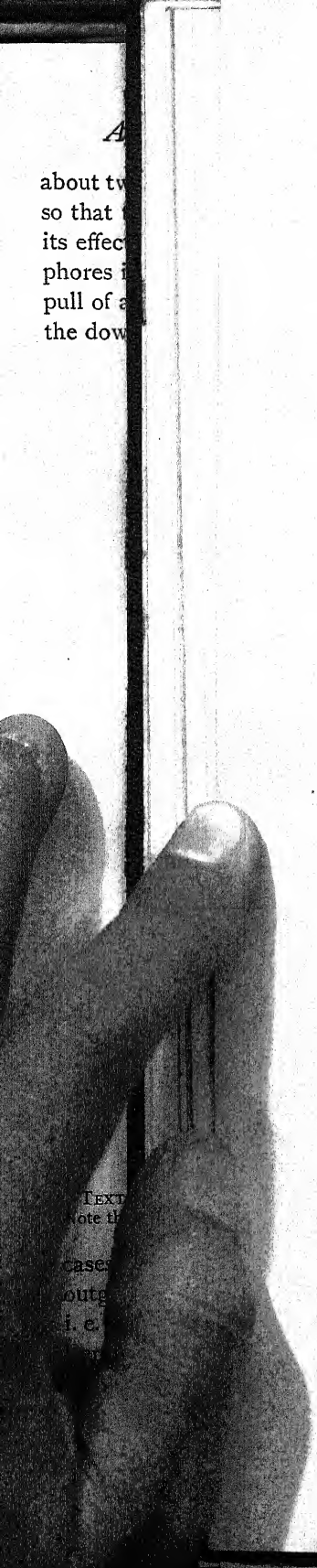
14.

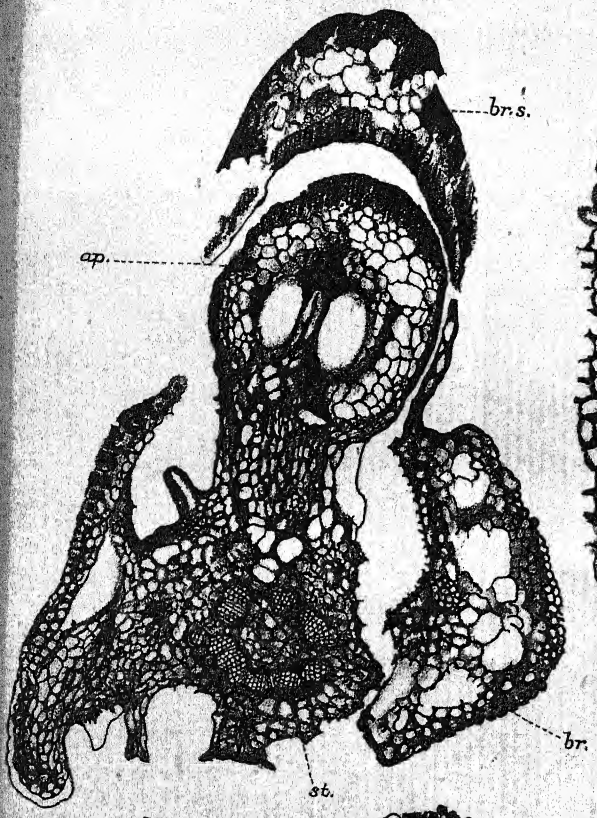
15.



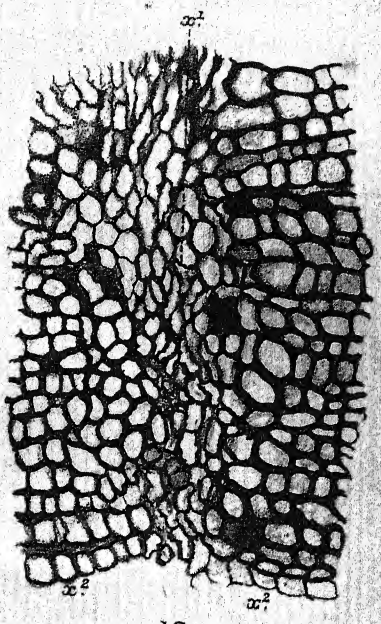
16.

Huth, coll.

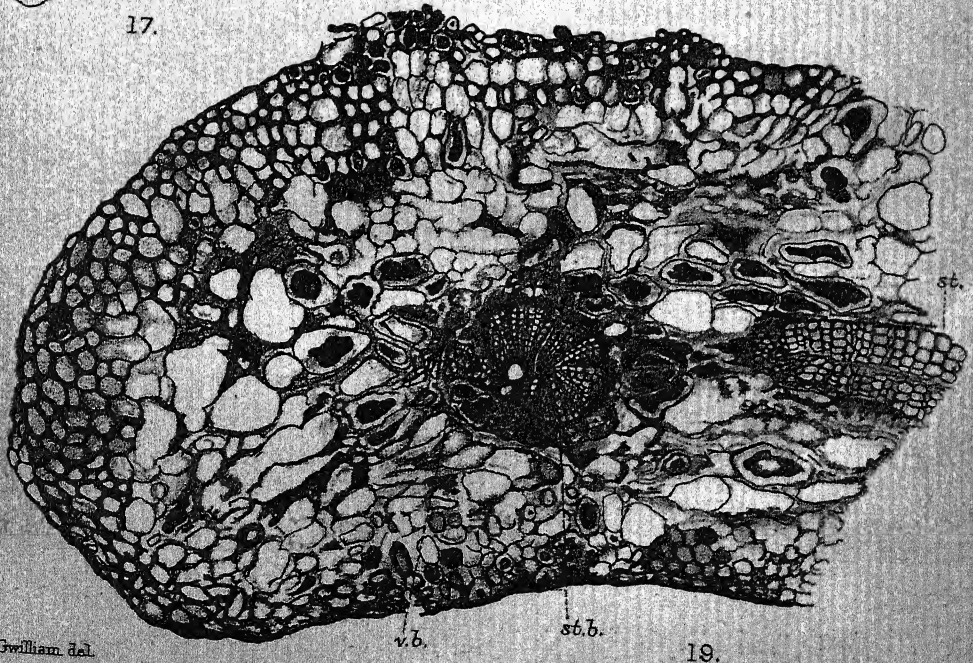




17.



18.



19.

G.T. Gwilliam. del.

SCOTT — MESOXYLON.

Huth. lith. et imp.

The Flora of Stewart Island (New Zealand): a Study in Taxonomic Distribution.

BY

J. C. WILLIS, M.A., Sc.D.,

European Correspondent, Botanic Gardens, Rio de Janeiro.

With two Maps and fourteen Tables in the Text.

IN this paper I shall put together a few notes upon the flora of Stewart Island, the southernmost of the main chain of the New Zealand islands proper. I have carried on this study by the aid of my hypothesis of age and area, which indicates many new directions in which to search for facts that have hitherto passed unnoticed. My former predictions as to composition and distribution made by its aid have been so successful, that in this paper I have adopted entirely the method of prediction and subsequent verification, giving fourteen different predictions about the plants of Stewart Island. The composition of the flora is first dealt with, and its geographical and other relationships. The subject of the probable invasions of New Zealand by plants, commenced in my last paper, is then followed up, in so far as the relationship of Stewart Island to these invasions is concerned, and it will be continued in papers on the floras of the more outlying islands.

In publishing this series of papers on the geographical distribution of the New Zealand flora I am sometimes accused by botanists in Europe of trespassing upon a line of work which various New Zealand botanists have made peculiarly their own. These investigators have a real knowledge of the local conditions, whereas I possess none, and are turning out work of great value and importance, upon which I should not dream of trespassing. But I may be permitted to point out that their work is concerned with ecological, not with taxonomic, distribution, and it is to the latter that age and area refers. The two lines of work are really very distinct, and have comparatively little overlap. What is beginning to come out with great clearness from the study of age and area is that, given fairly uniform conditions, such as one finds in New Zealand, the distribution of the flora about the country, so far as the area occupied by the different species is concerned, is governed almost entirely by the law of age and area. The

[Annals of Botany, Vol. XXXIII. No. CXXIX. January, 1919

mere fact that one can make so many predictions about any of the floras of the New Zealand region, and find, on verification of the facts, that they are justified, is alone sufficient to show this. The principal cause that interferes with the uniformity of the action of age and area is the presence of actual barriers, such as Cook's Strait, Foveaux Strait, the central mountain chain, the sea dividing New Zealand from the outlying islands, and so on, but within New Zealand the actual ecological barriers, which might easily alter very largely the distribution of species if they were of sufficient breadth and width, do not seem to affect the question of area occupied, save in quite a minor degree. The plants are locally distributed, within the area which is assigned to them by the passage of time, in accordance with their reactions to the various ecological factors which are operative there. But ecology seems little concerned, so far as we can see, and so far as the figures of distribution give any guide, with the actual composition of the flora. Unfavourable ecological conditions may determine that a certain species shall not survive in a given place, so that it may reach what, in a list of modifying causes we have given in a previous paper (6, p. 206), we have termed the climatic boundary, but which might better perhaps be termed in a more general way the ecological boundary. But otherwise ecology simply seems to make what it can, so to speak, of the floras with which it is provided by the mere action of phylogenetic descent and of time.

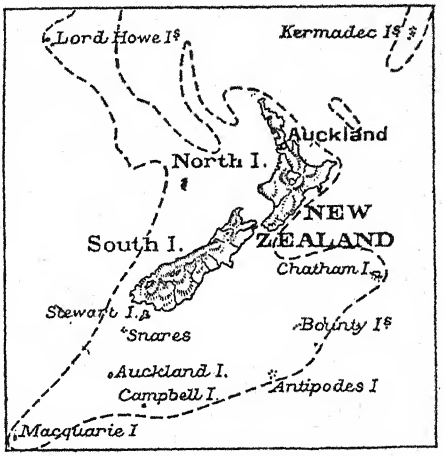
That the composition of the flora of Stewart is what it is, is largely due to the simple fact that certain species were at a certain distance from Stewart early enough to arrive there before the formation of Foveaux Strait, which cut it off from New Zealand proper. The figures of distribution give little evidence to show that many species have arrived since the formation of the strait, though there are a few, for example *Urtica australis*, which seem to be such cases. In general the distribution follows age and area with such closeness that one may make predictions on this basis alone, and find them often within perhaps five per cent. of accuracy.

It is worth while pointing out that by using the hypothesis of age and area one finds great numbers of new facts ready to be picked up, or explanations of what have hitherto been regarded as facts to be simply accepted as such. As one has as yet no guide as to their relative immediate importance, one must be content to collect them all, and no doubt they will in time prove their comparative values.

Stewart Island is a small island of 664 square miles, separated from South Island by Foveaux Strait, which at its narrowest has a breadth of sixteen miles, and at its shallowest (centre) a depth of fifteen fathoms (thirty metres). It would therefore seem not unreasonable to suppose that the separation from South Island was at a very remote period. This is confirmed by the fact that Stewart possesses local endemic species, confined

to itself alone. In its relations to South Island it occupies a position like that of Ceylon in relation to South India.

It has the same indented rocky coast as South Island, and is similarly mountainous, Mt. Anglem reaching 3,200 feet. Geologically it is chiefly composed of archaean rocks, like those in the extreme south-west of South Island. It is clear from the soundings (see map) that it must have received its flora by way of New Zealand proper, and, being closer to the main islands, and probably not divided from New Zealand at an earlier period than the Chathams and Aucklands, it has received many more plants than they, and has double the flora of either of them.



New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

Stewart Island being thus away from the general centres of distribution of the New Zealand flora, which in the previous paper we have seen to have been, one probably in the North Island, the other somewhere about the middle of the southern half of the South Island, a brief consideration of its relationships from the standpoint of age and area enables us to say that its flora will be composed of species which in comparison to those of New Zealand proper will be *old*.[1] We shall work upon this as a fundamental fact and go on to study the flora by the method of prediction and verification.

Composition of the Flora of Stewart Island.

As showing that age and area lends itself to prediction, we shall begin by endeavouring to predict as far as may be the actual composition of the flora, assuming that we know nothing about Stewart other than its position, but that we know the floras of New Zealand and the other outlying islands to the extent described by Cheeseman (1, 2).

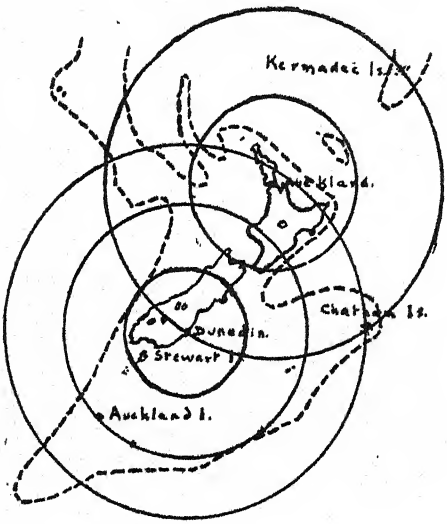
To commence with the actual size of the flora : we have seen that the falling off in number of species from their zones of greatest concentration is fairly regular (8, p. 343[2]), and we should expect, if Stewart were in land connexion, that the flora would be about 550, but as the formation of the strait must have been early enough to prevent many species from crossing which have since reached the strait from the north, we shall not expect so

[1] The same conclusion follows almost as clearly from the usual interpretation of the hypothesis of Natural Selection.

[2] Species endemic to New Zealand and islands are not given in this list.

many, but perhaps 400 or less. In actual fact the flora is 311, to which must be added 57 ferns. (Cf. Appendix, p. 42.)

In my last paper we saw that New Zealand probably received the bulk of its flora by two great invasions, one northern, within 400 miles from the north end of North Island, and the other southern, with its centre somewhere a little south of the middle of South Island. If we assume, for the sake of simplicity, that Auckland and Dunedin were the actual centres of these invasions,[1] and draw circles round them, as done in the adjoining map, then it is clear that the older plants will be near the boundaries of the outer circles, in so far as actual barriers of sea have not interfered with their distribution, and the younger successively nearer to the centres. It therefore follows (this thesis will be more fully developed in a later paper on the outlying islands) that the Chatham Islands should have a good deal in common with the Kermadecs of the older families, genera, and species, which can have reached both, and similarly much in common with the Aucklands, while of families, genera, and species that are sufficiently old in New Zealand all three groups of islands should possess representatives. The circle for the northern invasion that covers the Kermadecs and Chathams does not include Stewart, while that for the southern invasion which covers the Chathams and Aucklands, or the Chathams and Campbell, does.



New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

The general result of this prediction, then, so far as concerns Stewart, is that one will expect to find there all families, genera, and species that occur in the Kermadecs, Chathams, and Aucklands, or in the last two only. But as Stewart is nearer to the centres, one may expect to find there also a good many that have not reached the Kermadecs and Aucklands, or the Chathams and Aucklands, or even any of the outlying islands.

The Chathams, in their intermediate position, will probably contain a good many plants which do not reach the Aucklands. A straight line drawn from them to New Zealand reaches it at a point which is much

[1] Examination of the map will show that displacement of the centres of the circles by 100 miles or so makes no difference to the arguments in this paper.

farther from Stewart than from the Chathams. But if we draw a line direct from Stewart to the Chathams, and use it as the diameter of a circle, this circle cuts New Zealand near Lake Taupo in the North Island, and near Foveaux Strait. One will therefore expect to find, to a large extent, that those species which occur in the Chathams, and reach Lake Taupo as well as Foveaux Strait, will occur in Stewart, unless that island was cut off too early, a point as to which we have no information.

The southern invasion, as we shall see in the next paper, probably passed not *very* far from the Aucklands, and a circle with its centre in Dunedin and passing through Stewart also passes through the north end of South Island. One will therefore expect to find most Auckland species that reach the north end of South Island also occurring in Stewart, again of course in so far as an early cutting off of Stewart may not have interfered.

A family will very rarely arrive in a country as a group of genera simultaneously; some will arrive sooner than others. *On the whole*, therefore, a family with several genera will be older in the country than one with one, and the same will be true of genera, those with many species being on the whole the older. Now as the Stewart flora is mainly composed of older forms, we shall not go far wrong if we predict that it will contain most of those families that contain more than the average number of genera (in New Zealand proper), and those genera that contain more than the average number of species. This then may be added to the previous predictions. As, however, there are not so many families and genera with numbers above the average, this will not, probably, add very many.

We shall now give a complete list of the flora of Stewart, marking against each species and family the result of applying to it these different predictions. Everything that is actually predicted is printed in italics. As a rule when a genus or family is predicted some of the actual species are also predicted, but in the few cases where this does not happen, the genus or family is printed in italics, the species in roman type.

TABLE I (and cf. p. 42).

A occurring in the Kermadecs, Chathams, and Aucklands, or the two latter.
B occurring in the Chathams, and reaching Lake Taupo and Foveaux Strait.
C occurring in the Aucklands, and reaching the north end of South Island.
Families with five or more genera, and genera with five or more spp., are marked with †.

	A	B	C
RANUNCULACEAE	×	×	×
Clematis † indivisa			
Ranunculus † Lyallii			
gracilipes			
hirtus		×	
Kirkii (endemic St)			
lappaceus			
rivularis		×	
acaulis	×	×	×
MAGNOLIACEAE			
Drimys colorata			
CRUCIFERAE †	×	×	×
Cardamine † *hirsuta*	×	×	×
Lepidium † *oleraceum*	×	×	×
tenuicaule			
VIOLACEAE		×	
Viola filicaulis			

	A	B	C
Viola Cunninghamii		×	
Melicytus ramiflorus			
lanceolatus			
Hymenanthera † crassifolia			
PITTOSPORACEAE			
Pittosporum † Colensoi			
CARYOPHYLLACEAE	×	×	×
Stellaria † *parviflora*		×	
Colobanthus † *Muelleri*		×	
Spergularia media			
PORTULACACEAE			×
Claytonia australasica			×
Montia fontana		×	×
ELATINACEAE			
Elatine americana			
MALVACEAE		×	
Plagianthus betulinus		×	
TILIACEAE			
Aristotelia racemosa			
fruticosa			
Elaeocarpus Hookerianus			
LINACEAE		×	
Linum monogynum		×	
GERANIACEAE	×	×	×
Geranium † *microphyllum*			×
sessiliflorum			
molle		×	
Pelargonium australe		×	
CORIARIACEAE		×	
Coriaria ruscifolia		×	
ROSACEAE	×	×	×
Rubus australis			
cissoides			
schmidelioides			
Geum † leiospermum			
Acaena † *sanguisorbae*	×	×	×
SAXIFRAGACEAE †			
Donatia novaezealandiae			
Carpodetus serratus			
Weinmannia racemosa			
CRASSULACEAE	×	×	×
Tillaea † *moschata*	×		×
diffusa			
DROSERACEAE			×
Drosera † *stenopetala*			×
Arcturi			
spathulata			
binata			
HALORRHAGIACEAE	×	×	×
Haloragis † alata			
tetragyna			
depressa			
micrantha			×
Myriophyllum elatinoides			
intermedium			
pedunculatum			
Gunnera † *monoica*		×	
Hamiltoni			
Callitriche Muelleri		×	
MYRTACEAE	×	×	×
Leptospermum scoparium		×	
Metrosideros † *lucida*			×
hypericifolia			
Myrtus pedunculata			

	A	B	C
ONAGRACEAE	×	×	×
Epilobium † *Billardierianum*		×	
pubens		×	
alsinoides		×	
rotundifolium		×	
linnaeoides			×
nummularifolium	×	×	×
Fuchsia excorticata			×
Colensoi			
FICOIDEAE		×	
Tetragonia expansa			
trigyna		×	
UMBELLIFERAE †	×	×	×
Hydrocotyle † tripartita			
americana			
novaezealandiae			
microphylla			
asiatica		×	
Actinotus novaezealandiae			
Apium prostratum			
Crantzia lineata		×	
Aciphylla † Traillii			
Ligusticum † intermedium			
flabellatum (endemic)			
ARALIACEAE †	×	×	×
Aralia Lyallii			
Panax † *simplex*			×
Edgerleyi			
Colensoi			
Schefflera digitata			
Pseudopanax † crassifolius			
CORNACEAE		×	
Griselinia littoralis			
RUBIACEAE	×	×	×
Coprosma † lucida			
rhamnoides			
parviflora			×
acerosa		×	
propinqua		×	
foetidissima	×	×	×
Colensoi			
cuneata			×
repens			×
Nertera depressa			×
dichondraefolia			
setulosa			
Asperula perpusilla			
COMPOSITAE †	×	×	×
Lagenophora † *Forsteri*	×	×	×
petiolata			
Brachycome † pinnata			
Thomsoni			
Olearia † angustifolia			
Traillii (endemic)			
Colensoi			
nitida			
ilicifolia			
avicenniaefolia			
Celmisia † Sinclairii			
petiolata			
longifolia			
linearis			
sessiliflora			
argentea			
Gnaphalium † *luteo-album*	×	×	×
japonicum		×	

	A	B	C
Gnaphalium † *collinum*		×	
Raoulia † Goyeni (endemic)			
Helichrysum † *bellidioides*	×	×	×
filicaule		×	
Cassinia † *Vauvilliersii*			×
fulvida			
Craspedia uniflora			
Cotula † *coronopifolia*		×	
australis		×	
Traillii (endemic)			
dioica			×
Abrotanella † muscosa (endemic)			
Erechtites † *prenanthoides*	×	×	×
arguta			
scaberula		×	
diversifolia			
glabrescens			
Senecio † bellidioides			
Lyallii			
lautus		×	
Stewartii (endemic)			
elaeagnifolius			
rotundifolius			
Microseris Forsteri			
Sonchus asper	×	×	×
oleraceus		×	
STYLIDIACEAE			
Phyllachne Colensoi			
Oreostylidium subulatum			
Forstera sedifolia			
GOODENIACEAE			
Selliera radicans			
CAMPANULACEAE †	×	×	×
Pratia angulata	×	×	×
Wahlenbergia saxicola			
ERICACEAE			
Gaultheria † antipoda			
perplexa			
EPACRIDACEAE †	×	×	×
Pentachondra pumila			
Cyathodes † acerosa			
empetrifolia			×
Leucopogon Fraseri			
Archeria Traversii			
Dracophyllum † Menziesii			
longifolium			×
Urvilleanum			
Pearsoni (endemic)			
rosmarinifolium			
PRIMULACEAE	×	×	×
Samolus repens	×	×	×
MYRSINACEAE	×	×	×
Myrsine † Urvillei			
divaricata			×
nummularia			
LOGANIACEAE			
Mitrasacme novaezealandiae			
GENTIANACEAE	×	×	
Gentiana † lineata			
Grisebachii			
saxosa			
Liparophyllum Gunnii			
BORAGINACEAE	×	×	
Myosotis † antarctica			
capitata			

	A	B	C
CONVOLVULACEAE †		×	
Calystegia Soldanella		×	
Dichondra brevifolia			
SCROPHULARIACEAE †	×	×	
Glossostigma elatinoides			
Veronica † *salicifolia*		×	
amabilis			
elliptica	×		
buxifolia			
Ourisia † macrophylla			
sessilifolia			
caespitosa			
Euphrasia † Dyeri			
LENTIBULARIACEAE			
Utricularia † monanthos			
PLANTAGINACEAE			×
Plantago † Raoulii			
Brownii			×
triandra			
CHENOPODIACEAE †		×	
Chenopodium † glaucum			
Atriplex Billardieri		×	
POLYGONACEAE	×	×	×
Rumex neglectus			×
Muehlenbeckia australis		×	
CHLORANTHACEAE			
Ascarina lucida			
THYMELAEACEAE		×	
Pimelea † Lyallii			
Drapetes Dieffenbachii			
Lyallii			
URTICACEAE †	×		
Urtica australis	×		
ORCHIDACEAE †	×	×	×
Dendrobium Cunninghamii			
Earina mucronata		×	
suaveolens			
Sarcochilus adversus		×	
Thelymitra † *longifolia*	×	×	×
uniflora			×
Microtis porrifolia		×	
Prasophyllum Colensoi			
Pterostylis † *Banksii*		×	
australis			
graminea			
Lyperanthus antarcticus			×
Caladenia Lyallii			×
bifolia	×	×	×
Chiloglottis cornuta	×	×	×
Corysanthes † *oblonga*			×
rivularis			×
rotundifolia			×
triloba			×
macrantha	×	×	×
Gastrodia Cunninghamii		×	
IRIDACEAE		×	
Libertia ixioides		×	
pulchella			
LILIACEAE †	×	×	×
Rhipogonum scandens		×	
Enargea marginata			
Cordyline † australis			
Astelia † *linearis*			×
nervosa			

	A	B	C
Phormium tenax	×	×	×
Bulbinella Hookeri			
Arthropodium candidum			
Herpolirion novaezealandiae			
JUNCACEAE	×	×	×
Juncus † pallidus			
effusus			
bufonius	×	×	×
planifolius	×	×	×
antarcticus			×
novaezealandiae			
Luzula † *campestris*	×	×	×
NAIADACEAE †		×	
Triglochin striatum		×	
Potamogeton † polygonifolius			
Cheesemanii			
Zostera nana			
CENTROLEPIDACEAE			×
Centrolepis viridis			×
Gaimardia setacea			
RESTIONACEAE		×	
Leptocarpus simplex		×	
Hypolaena lateriflora		×	
CYPERACEAE †	×	×	×
Eleocharis † sphacelata			
Scirpus † *aucklandicus*			×
cernuus	×	×	×
antarcticus			
inundatus		×	
nodosus		×	
Carpha alpina			×
Schoenus † pauciflorus			
axillaris		×	
nitens			
Cladium † *glomeratum*		×	
Gunnii			
Vauthiera			
Gahnia † procera			
Oreobolus pumilio			×
strictus			
Uncinia † caespitosa			
australis	×	×	×
leptostachya			
riparia			×
rupestris			
filiformis			
Carex † *appressa*	×	×	×
echinata			
ternaria			×
testacea			
lucida			
uncifolia			
comans			
litorosa			
dissita			
Solandri			
longiculmis (endemic)			
trifida			×
GRAMINEAE †	×	×	×
Ehrharta Thomsoni			
Microlaena stipoides			
avenacea			×
Hierochloe redolens	×	×	×
Fraseri			

	A	B	C
Deyeuxia † *Forsteri*	×	×	×
setifolia			×
avenoides			
quadriseta			
Dichelachne crinita		×	
Deschampsia † *caespitosa*	×	×	×
Trisetum antarcticum		×	
Danthonia † Cunninghamii			
Raoulii			
Crassiuscula			
pungens (endemic)			
pilosa			
semiannularis		×	
Arundo conspicua		×	
Poa † foliosa			
novaezealandiae			
Astoni			
seticularis			
pusilla			
caespitosa			
Colensoi			
imbecilla		×	
Atropis novaezealandiae			
Festuca † *littoralis*		×	
rubra			
FILICES			
Hymenophyllum † *rarum*	×	×	×
polyanthos			×
villosum			×
australe			
pulcherrimum			
dilatatum	×	×	×
demissum	×	×	×
flabellatum	×	×	×
rufescens			
subtilissimum			
Cheesemanii			
minimum			×
tunbridgense			×
unilaterale			
multifidum	×	×	×
bivalve		×	×
Trichomanes † *reniforme*		×	
Lyallii			
venosum		×	
strictum			
Cyathea medullaris		×	
Hemitelia Smithii			×
Alsophila Colensoi			
Dicksonia squarrosa		×	
Lindsaya linearis		×	
Adiantum † *affine*		×	
Hypolepis tenuifolia		×	
Pteris † *aquilina*	×	×	×
scaberula		×	
incisa	×	×	×
Lomaria † (*Blechnum*) *Patersoni*			×
discolor	×	×	×
vulcanica			
lanceolata		×	
dura	×		
Banksii			
alpina		×	
capensis	×	×	×
fluviatilis		×	×
Asplenium † *adiantoides*		×	

	A	B	C
Asplenium obtusatum	×	×	×
lucidum	×	×	×
bulbiferum		×	
flaccidum	×	×	×
Polystichum † *aculeatum*	×	×	×
capense		×	
Dryopteris † *hispida*		×	
Polypodium † *punctatum*	×	×	×
Billardieri	×	×	×
Polypodium † *grammitidis*	×	×	×
Cyclophorus serpens		×	
Gleichenia † circinata			
dicarpa		×	
Cunninghamii			
Schizaea fistulosa	×	×	×
Todea hymenophylloides			
superba			×

A glance at the table will show that the species predicted form a very representative well-distributed assortment. There are 70 out of 200 Dicotyledons, or 35 per cent., 53 out of 111 Monocotyledons, or 47 per cent., and 43 out of 57 ferns, or 75 per cent. We may draw attention to Onagraceae, and especially *Epilobium* (all 6 species predicted), *Coprosma* (6 of 9), Orchidaceae, Juncaceae, and the ferns, including *Pteris* (all 3 species), *Lomaria* (7 out of 9), and *Asplenium* (all 5).

There remain unpredicted 188 Angiosperms and 14 ferns. Ten of the former are local endemics which are dealt with below, and it might be possible to maintain that there is still room for relicts among the remaining 178. It is therefore of interest to trace their distribution in New Zealand. None have discontinuous areas, which is a feature that one might expect sometimes to show among relicts. Classifying them according to their range (two do not occur in New Zealand proper) we get :

TABLE II.

		No. of Species.		*Including* 33 wides	42 endemics[1]
Class 1.	1,001–1,080 m.	75		33	42
2.	881–1,000	16		3	13
3.	761–880	26	146	10	16
4.	641–760	14		3	11
5.	521–640	15		1	14
6.	401–520	14		2	12
7.	281–400	4		—	4
8.	161–280	7	30	—	7
9.	41–160	5		—	5
10.	1–40	—		—	—
		176		52	124
Mean rarity		3·0		1·8	3·4
Mean rarity for New Zealand		5·5		3·7	6·1

It is fairly evident that one can hardly consider as relicts the 146 species of classes 1 to 5, which also reach the North Island, and that include 50 wides, some of which, like *Spergularia media*, are almost world-ranging. Thus there remain at most only 30 to choose from, or less than 10 per cent. of the flora of Stewart. It would be stretching too far an unproved hypothesis (that of the supposition that most species of very limited range are dying out) to include the 14 in class 6, which are mostly held up at

[1] Endemic to New Zealand or New Zealand and islands.

Cook's Strait, and which include two wides, so that there really only remain 16, in classes 7 to 9. Of the 5 in class 9, 4,[1] *Gunnera Hamiltoni*, *Olearia angustifolia*, *Veronica amabilis*, and especially *Atropis novaezealandiae*, are more or less closely confined to the coast, and I must leave it to the ecologists to settle whether they can be in any way regarded as relicts, or whether they are really coast species which arrived (or evolved) late, and by water carriage.

In any case, however, these last 16 species belong all of them to fairly large (old) genera (i. e. large in number of species in New Zealand). The genera of the 5 in class 9 contain in New Zealand 147 species, an average of almost 30; those of the 7 in class 8 contain 140 species, or an average of 20; those of class 7 are 4 with 38 species, or an average of 9. All are much above the average size of genera in New Zealand (4·2 species). It is obvious that these species belong to the same class of genera (the larger and older) as do the actual endemics of Stewart itself, which are dealt with below (10 genera, 249 species). As one approaches the centre of New Zealand one finds endemics in increasing numbers belonging to smaller and smaller genera, which, as we have seen above, are on the whole younger and younger genera. If these are relicts, then it is clear that the younger and smaller (in the country) a genus is, the greater its chance of giving rise to relicts; but the very awkward problem at once crops up, to explain why the relicts are crowded together in the centre of the main country of New Zealand, where there are also the most wides, as we have already shown (6, p. 201). There is not, it seems to me, any evidence about these species to give any ground for supposing them relicts.

A comparison of the figures of rarity given at the bottom of Table II will also prove of little encouragement to the upholders of the hypothesis of dying out. All these species are on the average far more wide-ranging in New Zealand than the averages of the groups to which they belong. Even the endemics show a greater average range in New Zealand than the average of the *wides* of that country (7, p. 331).

Examination of the 14 unpredicted ferns (where if anywhere one might expect relicts) gives even less cause to believe in extinction. Six belong to class 1, ranging New Zealand from end to end, 3 to class 2, 5 to class 3, and none to any more localized class.

There yet remains one objection which may be brought up against the whole prediction and discussion given above. It may be said that I have drawn my predictions so wide that I have included the whole flora, and have simply picked out from this list the species belonging to Stewart. As the bulk of the predictions are based upon the actual floras of the other islands, this can hardly be the case, but it will be well in conclusion to give a list of

[1] The fifth is *Gentiana lineata*, occurring in Longwood Range and Blue Mountains, Otago, and an unknown locality in Stewart.

the species predicted for Stewart that are not recorded. Under prediction *A* everything was exact. Under *B* (species reaching the Chathams, Lake Taupo, and Foveaux Strait) the following have not been found in Stewart, though predicted as likely:

Plagianthus divaricatus (cf. p. 42)
Geranium dissectum
Sophora tetraptera
Potentilla anserina (cf. p. 42)
Epilobium pallidiflorum (cf. p. 42)
„ chionanthum
„ insulare (cf. p. 42)
Mesembryanthemum australe (cf. p. 42)
Hydrocotyle moschata
Oreomyrrhis andicola (cf. p. 42)
Daucus brachiatus
Coprosma robusta
„ Cunninghamii
Erechtites quadridentata (cf. p. 42)
Lobelia anceps
Wahlenbergia gracilis (cf. p. 42)
Myosotis spathulata (cf. p. 42)
Calystegia tugoriorum (cf. p. 42)
Dichondra repens
Solanum nigrum
„ aviculare
Myoporum laetum
Mentha Cunninghamii (cf. p. 42)
Salicornia australis
Polygonum serrulatum
Pimelea arenaria
Scirpus frondosus (cf. p. 42)
„ americanus
Deyeuxia Billardieri (cf. p. 42)

These are twenty-nine in all, of which sixteen are wides, and there is no reason to suppose that their absence from Stewart is due to anything other than the fact that Foveaux Strait was formed before they reached so far south in their wanderings. It is noteworthy that the list contains only three Monocotyledons, though this group forms so large a proportion of the flora of Stewart (111 of 311).[1]

Finally, under prediction *C* (Auckland species reaching to Foveaux Strait and the north end of South Island) there were wrongly predicted:

Colobanthus Billardieri (cf. p. 42)
Epilobium confertifolium
Rostkovia gracilis

to which the same explanation may apply.

General Features and Relationships of the Flora.

We shall now go on to apply the method of prediction in greater detail, with the view of bringing out the general features and relationships of the flora of Stewart.

(1) As the family is older than the genus, the genus than the species, one will expect that in comparison with the flora of New Zealand proper a greater proportion of families will be represented than of genera, of genera than of species. Verification of this gives:

[1] In a later paper I hope to deal with the distribution of the Monocotyledons, which shows many interesting points, e. g. a regularly diminishing percentage from one end of New Zealand to the other, and twice as great a percentage in the Aucklands as in the Kermadecs, with an intermediate figure in the Chathams, and so on.

TABLE III.

	Families.	*Genera.*	*Species.*
New Zealand	91	329	1,392
Stewart	54 or 59 %	154 or 46 %	311 or 22 %

(2) A family will rarely arrive as a group of genera simultaneously; some will arrive sooner than others. Therefore, on the whole, families with more than one genus in a given country will be older there than those with only one in that country, and may be expected to be better represented. Testing this, we get :

TABLE IV.

Family represented in New Zealand by	*In New Zealand.*	*Represented in Stewart by*		*Not represented there.*
1 genus	36 families	13 families,	36 %	23 families
2	15	6	40	9
3	15	12	80	3
4–5	10	9	90	1
6–10	9	8	90	1
over 10	6	6	100	–
	91	54	59 %	37

In other words, the most 'successful' families in New Zealand are the best represented in Stewart, and the proportion of families shows a steady increase with the increasing number of genera contained in them.

One may even push this into greater detail, and take the thirty-six families with one genus, dividing them according to the number of species in the genus, when one finds:

TABLE V.

Genera.	*No. of Species contained.*	*Represented in Stewart by*	
7	6 or more; 50 in all	14 species, or 28 %	
5	3, 4, or 5; 17	4	23
24	1 or 2 ; 30	3	10

(3) One may extend the idea indicated in this last table, and make the same prediction about the genera as about the families, and say that those with most species in New Zealand will be the best represented in Stewart.

TABLE VI.

Genus represented in New Zealand by	*In New Zealand.*	*Represented in Stewart by*		*Not represented there.*
1 species	155 genera	32 genera, or 20 %		123 genera
2	54	22	40	32
3	29	20	68	9
4–5	29	23	79	6
6–10	36	32	88	4
11–20	16	15	93	1
over 20	10	10	100	—
	329	154	46 %	175

Thus, just as with the families, the proportion of genera represented in Stewart shows a steady increase with the increasing number of species in the genus, from 20 per cent. of those with one up to 100 per cent. of those with more than 20 species.

Of the 311 species of the flora of Stewart, 206, or 66 per cent., belong to the comparatively few genera that possess in New Zealand 5 or more species in the genus, the average number in a genus in New Zealand being 4·2.

On the hypothesis of Natural Selection, as usually interpreted, one would expect to find Stewart Island—an outlying island, small in area, simple in geological structure, with small flora and peopled by old species—occupied largely by plants unsuited to life in the crowded South Island, in fact a kind of refuge for the destitute. This expectation is vehemently contradicted by what we have just indicated. The genera of Stewart, as a glance at the list of the flora will show, are in reality what it has been the custom to call the 'successful' genera of the neighbouring island, from which it must have received its flora. These genera are in reality simply those which were the first to arrive of their various affinity groups. The first 10 genera of the Stewart flora are represented in New Zealand by 103 species, or an average of 10·3 species per genus, against an average for New Zealand of 4·2. The last 10 have 70 species in New Zealand, or an average of 7·0. The 154 genera of Stewart contain in New Zealand 1,067 species, against 325 for the 175 unrepresented genera.

(4) The Stewart Island plants being old, we shall expect to find 'wides', which are the oldest forms, best represented among them.

TABLE VII.

	Wides.	*Endemic to New Zealand or New Zealand and Islands.*
New Zealand as a whole	301	1,000
Stewart Island	111, or 36 %	187[1], or 18 %

(5) One will expect to find the Stewart Island plants, as very old, very widespread in New Zealand:

TABLE VIII.

Class.	*Range in New Zealand.*	*Wides.*	*Endemic to New Zealand and Islands.*	*Endemic to New Zealand only.*
1	1,001–1,080 miles	81	37	48
2–4	641–1,000	23	18	40
5–10	1–640	7	8	36
		111	63	124

Thus 166 plants, or 55 per cent., belong to the first class, which ranges New Zealand from end to end. Even the species endemic to New Zealand alone show more in the first class than in classes 2, 3, and 4 put together, though in New Zealand as a whole the numbers in the first four classes are, 52, 60, 59, and 61.

If we calculate out the rarity in the usual manner, we find that while in

[1] The remaining thirteen include the ten local endemics, and *Aralia Lyallii*, *Urtica australis*, and *Poa foliosa*, not found in New Zealand proper, and perhaps, or probably, water-borne.

New Zealand as a whole the wides show a rarity of 3·7, those that occur in Stewart show 1·7. As each unit of rarity represents 120 miles, this means that they range (in New Zealand) on the average 240 miles farther. Similarly the species endemic to New Zealand and the islands show 2·2 for Stewart against 2·9 [1] for New Zealand as a whole, and the species endemic to New Zealand only show 3·2 against 6·5. In other words, they range on an average 796 miles against 400, and even exceed in their range the average *wide* of New Zealand as a whole, which ranges only 736 miles. This is a feature which is quite impossible of explanation by aid of the doctrine of Natural Selection.

(6) As the ferns are probably older, Stewart should have proportionately more of them than New Zealand proper, but one must remember that the question of the ferns is complicated by the probability—amounting to practical certainty—that new wides may continue to arrive long after the barrier interposed by the sea has become practically impassable to more than a very few Angiosperms (8, p. 340).

TABLE IX.

	Wides.		*Endemic to New Zealand and Islands.*		*Endemic to New Zealand only.*	
	Ferns.	*Angiosperms.*	*Ferns.*	*Angiosperms.*	*Ferns.*	*Angiosperms.*
New Zealand	92	301	12	98	24	902
Stewart	39, or 42 %	111, or 36 %	8, or 66 %	63, or 64 %	10, or 41 %	124, or 13 %

All the figures for ferns are higher than for Angiosperms, though there is considerable difference among them. The difference, however, is most marked in the New Zealand endemics, as would be expected.

(7) Stewart Island possesses 10 species endemic to itself alone, and found nowhere else. As on the whole, as we have already pointed out, the largest families in New Zealand will be the oldest, we shall expect these endemics to belong to them (and cf. Appendix, p. 42).

TABLE X.

Family (in order of size in New Zealand).	*Species in New Zealand.*	*Species in Stewart.*	*Endemics.*
1. Compositae	203	44	*Olearia Traillii* *Raoulia Goyeni* *Cotula Traillii* *Abrotanella muscosa* *Senecio Stewartii*
2. Cyperaceae	117	34	*Carex longiculmis*
3. Scrophulariaceae	109	9	,,
4. Gramineae	97	30	*Danthonia pungens*
5. Orchidaceae	57	21	*Ligusticum flabellatum*
6. Umbelliferae	54	11	,,
7. Ranunculaceae	45	8	*Ranunculus Kirkii*
8. Rubiaceae	43	13	,,
9. Onagraceae	31	8	,,
10. Epacridaceae	28	10	*Dracophyllum Pearsoni*
Total 10 families	784 species	188 species	10 species

[1] For the reason why these species are more common than the average of the wides, see 7, p. 331.

Thus all the endemics are to be found in the 10 largest families in New Zealand, and as a matter of fact in 6 of them, which contain in New Zealand 544 species (total for New Zealand, 91 families and 1,301 species), and in Stewart 137 species (total for Stewart, 54 families and 311 species). They belong, that is, to the most 'successful' families in New Zealand. The great proportion belonging to Compositae is noticeable, and goes to indicate, with other parallel evidence that could be produced, that species are somewhat readily formed in this family.

(8) In the same way, one will expect the Stewart endemics to belong on the whole to the larger genera of New Zealand, and testing this, we find that *Olearia* has 35 species, *Raoulia* 17, *Cotula* 19, *Abrotanella* 7 (the nearest approach to the average of 4·2), *Senecio* 30, *Carex* 54, *Danthonia* 13, *Ligusticum* 18, *Ranunculus* 38, and *Dracophyllum* 18, a total of 249 species for 10 genera, or practically 25 each, almost six times the average for a genus in New Zealand.

Relationships of the Flora to those of the Outlying Islands.

We shall now go on to deal with the relationship of the flora of Stewart to those of the Kermadecs, Chathams, and Aucklands, which (see maps) are islands outlying much farther from New Zealand proper, but at the same time islands that must have received the bulk of their flora either by way of New Zealand, as in the case of the Chathams more especially, or at any rate from the same invasions, as in the case of the Kermadecs (to some extent) and the Aucklands. As authority for these floras I shall continue to use Cheeseman's Flora (1), supplemented by his later lists in Chilton's 'Subantarctic Islands of New Zealand' (2).

Stewart Island lies in lat. 47° S., the Aucklands in 51°, the Chathams in 44°, and the Kermadecs in 30°. The distance in a straight line from Stewart to the Aucklands is about 200 miles, to the Chathams about 700 miles, and to the Kermadecs about 1,200 miles, including nearly the whole of New Zealand, which lies between. Stewart is chiefly archaean rocks, the Aucklands all of igneous origin (some parts granites and gabbros, but mostly volcanic), the Chathams schists, volcanic rocks, or tertiary sediments, and the Kermadecs entirely volcanic (4).

(9) It is clear from the maps, especially the second map on p. 26, that while the plants of Stewart Island are old in New Zealand, those of the other more outlying islands will be older, in general, whether they were derived from New Zealand, or whether they reached these islands on the way to New Zealand. One will therefore expect to find that Stewart has a large proportion of species in common with these different islands, and that they have a larger proportion of species in common with Stewart than they have with New Zealand as a whole, though in general they are nearer to New Zealand. Testing this, we find:

TABLE XI.

Common to Stewart and Aucklands	71	species, or 22	% of flora of	Stewart
,, New Zealand and A.	83	6		New Zealand
,, Stewart and Chathams	84	27		Stewart
,, New Zealand and C.	118	9		New Zealand
,, Stewart and Kermadecs	22	7		Stewart
,, New Zealand and K.	52	3		New Zealand

That Stewart Island should have a great deal in common with the Aucklands might be expected, but that it should have proportionately so much more in common with the Chathams and the Kermadecs than has New Zealand, though the latter divides it from the Kermadecs, and is nearer to the Chathams, would never be expected on the older views as to distribution of species. This subject will be followed up in a subsequent paper on the outlying islands.

(10) On examination of the map, it will be noticed that no circle can be drawn to include the Chathams and Aucklands without including Stewart, unless it be placed with its centre in an impossible depth of soundings. Unless, therefore, the times of separation from New Zealand of Stewart and these islands were *very* different, we shall expect to find that all species common to the Chathams and Aucklands also occur in Stewart:

TABLE XII.

	Common to Chathams and Aucklands.	*Occur in Stewart.*
Dicotyledons	19	19
Monocotyledons	13	13
Ferns	19	19
	51	51

(11) If one draw a straight line from Stewart to the Chathams, bisect it, and use the point of bisection as the centre of a circle whose circumference passes through the islands mentioned, then it is evident that if at the time of dispersal of most species there was continuous land where the 1,000 fathom line now runs, the species common to Stewart and the Chathams should in general have covered this circle, or so much of it as was above water. The circle cuts the North Island near Lake Taupo, at about 350 miles from the North Cape, so that, except in so far as barriers caused by submergence of parts of the land, or in other ways, have interfered, we should expect these species to range in New Zealand up to or beyond Lake Taupo.

Examination soon shows that, of the 84 species common to Stewart and the Chathams, no less than 74 range New Zealand from end to end, while six more range from Stewart up to from 100 to 200 miles beyond Lake Taupo. This leaves only four, or less than 5 per cent., which do not reach the lake. These are *Tillaea moschata* and *Veronica elliptica*,

both coast species which may have arrived later than the rest, by water transport; *Carex appressa*, which is given by Kukenthal (3) as occurring throughout New Zealand, and which consequently will not be an exception, either here or in the previous paper in which it was given as such (7, p. 329); and finally *Pterostylis australis*, which is regarded by Hooker as a variety of *P. Banksii*, whose range is the entire length of New Zealand.

Of the ferns found in the Chathams, and reaching to Lake Taupo one way and Foveaux Strait the other, 36 reach Stewart and 7 reach only to the strait, whilst *Lomaria dura*, which reaches only about half-way along the South Island, though it occurs in Stewart, the Snares, the Aucklands, Campbell, and the Antipodes, as well as the Chathams, is given by Cheeseman as 'a purely littoral plant, never found far from the influence of sea-spray'.

(12) It is clear that of the many species which Stewart has in common with the other islands, those in common with the Kermadecs, which are the farthest away, will on the whole be the oldest, those in common with the Chathams probably next, and those in common with the Aucklands youngest. In other words, the first named should be the most widespread in New Zealand:

TABLE XIII.

Class.	*Range.*	*Stewart and Kermadecs.*	*Stewart and Chathams.*	*Stewart and Aucklands.*
1	1,001–1,080 m.	22, or 100 %	74, or 87 %	40, or 56 %
2	881–1,000	—	3	5
3	761–880	—	3	8
4	641–760	—	—	8
5	521–640	—	2	4
6	401–520	—	1	3
7	281–400	—	—	—
8	161–280	—	1	3
9	41–160	—	—	—
10	1–40	—	—	—
		—	—	—
		22	84	71
	Rarity (Classes 1–10)	1·0	1·3	2·3

Thus the Kermadec species have the maximum commonness (distribution area) possible, ranging New Zealand from end to end. The Chatham species are almost as common, and the Auckland species a good deal less so.

(13) As the Kermadec species are the oldest, they should include the greatest proportion of wides, while the species endemic to New Zealand and the islands, which are younger, should show more in the other islands:

TABLE XIV.

Common to	*Wides.*		*Endemic to New Zealand and Islands.*	
Stewart and Kermadecs	19, or 86 %		3, or 14 %	
„ Chathams	50	59	34	41
„ Aucklands	26	37	45	63

The Relationship of Stewart Island to Invasions of Plants.

We have now to go on to consider the relationship of Stewart Island to the great invasions of plants into New Zealand which were considered in my last paper (9, p. 355). And first let us deal with the northern invasion, which probably entered, we saw, at some point (or more likely space) in the North Island not more than 400 miles south of North Cape (the island is 520 miles long).

It is at once evident that the northern invasion was very early, or that it found New Zealand at the time of its entry comparatively unobstructed with vegetation, for although it had to travel the whole length to Foveaux Strait, 1,000 miles south of North Cape, none the less a very fair proportion of its species arrived there in time to cross to Stewart before it was too late. There reach the strait, of the plants of this invasion, 5 wides and 32 endemics, and of these 3 wides and 14 endemics cross the strait. Those which cross belong, as one would expect from predictions 2, 3, to the largest genera and families of the invasion. As a matter of fact they belong to Pittosporaceae (1 genus, 19 species), Saxifragaceae (6/8), Myrtaceae (4/18), Araliaceae (5/15), Cornaceae (2/5), Lentibulariaceae (1/6), and Urticaceae (5/10), a total of 7 families, 24 genera, and 81 species, i. e. an average per family of 3·4 genera, and per genus of 3·3 species. The remainder of the invasion consists of 26 families, with 38 genera and 52 species, averages of 1·4 genera per family and 1·3 species per genus.

To go on to the southern invasion, it is at once evident that this was either much later in New Zealand or found the ground more troublesome to traverse, as although it was mainly herbaceous, and we have some reason to suppose that herbs may travel faster than trees, of which the northern invasion was chiefly composed, and although it had its centre about 400 to 600 miles south of the other invasion, and was therefore so much the nearer to Stewart, none the less it is not so well represented there as the northern invasion. Only 51 wides out of 91 (at Foveaux Strait) occur in Stewart, or 56 per cent., against 3 out of 5, or 60 per cent., of the northern wides; and of the total of plants of the invasion, and its result in endemics, only 115 out of 286 cross Foveaux Strait, or 40 per cent., against 17 out of 37, or 45 per cent.

Had the southern invasion entered New Zealand by way of Stewart Island, one would not expect such a result, but would expect to find more species in Stewart than on the northern side of the strait. One may without any further discussion, it seems to me, come to the conclusion that the southern invasion of plants entered Stewart from the north side of Foveaux Strait, and consequently that Stewart did not lie in the direct track of that invasion.

Again it happens that (as predicted for the northern invasion) Stewart contains chiefly representatives of the larger genera—all the families of the southern invasion are to be found. It contains 56 genera of this invasion, represented in New Zealand by 451 species, against 52 unrepresented genera with 91 species.

We have thus made quite a number of predictions, based solely upon the hypothesis of age and area, about the flora of Stewart Island, and every one of them has proved to be correct, with a large margin. It is clear that it is fairly safe to make predictions from age and area; in other words, the operation of this law is by far the principal factor in determining the actual geographical distribution of species about the globe prior to the advent of man. Physical barriers of course have a much greater influence in determining the actual areas occupied, but their influence is of purely negative kind, while age is positive. Local distribution, in a given district, on the other hand, is determined by the ecological conditions of that district, but unless the ecological boundary (beyond which a species cannot grow) is fairly broad and wide, it does not seem to affect seriously the total area occupied within the outer limits.

It must again be made clear, for this is a point on which many of my critics fail to understand my position, that the law *must not* be applied to individual species, but only to groups of allied forms. It is a law which applies to taxonomic distribution, and its operations can only be clearly made out, and disentangled from the many other causes that aid in determining geographical distribution, by taking a group of allied species. After a lapse of x years a group of 20 herbaceous Compositae will occupy an area z; after a lapse of y years a group of arboreous Dipterocarpaceae will occupy the same area z in the same country, but we have no means of comparing x and y at present. Each, however, is governed by age and area.

Another point whose misunderstanding seems to cause difficulty for some in accepting the law, is the enormous differences between one species and another in the area occupied. They seem unable to conceive how this can come about without the operation of Natural Selection in reducing the area occupied by one of the species. In thinking over the subject they are apt to forget that the area occupied *per unit time* increases as the age of the species increases. So long as one only takes the diameter of the area occupied, as I have done in New Zealand, a plant when it reaches a diameter of, say, 1,000 miles is perhaps twice the age that it was when it reached 500. But if the species were spreading every way in untouched continental areas of uniform conditions, then, while the diameter increased

1 2 3 4 5 6 7

the area would increase

1 4 9 16 25 36 49

or, in other words, in equal times the increase in area occupied would be

3 5 7 9 11 13

so that it becomes quite easily possible for a species to spread over an enormous area once that its area occupied is large. Biologists who are not accustomed to working with figures are apt to forget that the area of a circle increases with the square of its diameter.

Appendix.

Since the above was in type, I have received from Dr. L. Cockayne, F.R.S., a most valuable letter of suggestion and criticism, and my one regret is that there is not time to think it over and incorporate the results in this paper, though I hope that further papers on New Zealand, which I have in preparation, will show its effects.

I had originally intended to base my statements in this paper solely on the work of Cheeseman (1, 2), but Dr. Cockayne has called my attention to the fact that his report on Stewart Island (10) contains many additions to the flora. There are so many that it would be unpardonable to ignore it, though its incorporation really makes no difference to my final results, and does not affect in any way the correctness of the predictions here made.

The additions to the flora made in the paper referred to are:—

	A	B	C
RANUNCULACEAE			
Ranunculus † Crosbyi (endemic)			
Caltha novaezealandiae			
VIOLACEAE			
Hymenanthera † dentata			
CARYOPHYLLACEAE			
Colobanthus † *Billardieri?*			×
HYPERICACEAE			
Hypericum japonicum			
MALVACEAE			
Plagianthus divaricatus		×	
TILIACEAE			
Aristotelia Colensoi			
CORIARIACEAE			
Coriaria thymifolia			
ROSACEAE			
Potentilla anserina		×	
Acaena † *novaezealandiae*		×	
HALORHAGIACEAE			
Myriophyllum Votschii			
Gunnera † prorepens			
arenaria?			
ONAGRACEAE			
Epilobium † *pallidiflorum*		×	
junceum			
pictum			
insulare		×	
novaezealandiae			

	A	B	C
FICOIDEAE			
Mesembryanthemum australe		×	
UMBELLIFERAE †			
Azorella † Cockaynei (endemic)			
Oreomyrrhis andicola		×	
Ligusticum † aromaticum			
ARALIACEAE †			
Panax † anomalum			
RUBIACEAE			
Coprosma † rotundifolia			
areolata			
ramulosa			
retusa			
COMPOSITAE †			
Olearia † divaricata (endemic)			
virgata			
Celmisia † rigida (endemic)			
Gnaphalium † trinerve			
Raoulia † australis			
glabra			
Helichrysum † Loganii?			
grandiceps			
Abrotanella † linearis			
Erechtites † *quadridentata*		×	
Taraxacum glabratum			×
STYLIDIACEAE			
Phyllachne clavigera			
CAMPANULACEAE †			
Wahlenbergia gracilis		×	

	A	B	C
MYRSINACEAE			
Myrsine † chathamica (prob. introd.)			
APOCYNACEAE			
Parsonsia heterophylla			
BORAGINACEAE			
Myosotis † *spathulata*		×	
CONVOLVULACEAE †			
Calystegia tugoriorum		×	
SCROPHULARIACEAE †			
Veronica † Laingii (endemic)			
Ourisia † prorepens ?			
modesta (endemic)			
Euphrasia † repens			
LABIATAE			
Mentha Cunninghamii		×	
ILLECEBRACEAE			
Scleranthus biflorus			
POLYGONACEAE			
Muehlenbeckia complexa			
LORANTHACEAE			
Loranthus † micranthus			
EUPHORBIACEAE			
Euphorbia glauca			
LILIACEAE †			
Astelia † subulata			
Phormium Cookianum			
Bulbinella Gibbsii (endemic)			
JUNCACEAE			
Juncus † lamprocarpus			
CYPERACEAE †			
Eleocharis † *acuta*		×	
Cunninghamii			
Scirpus † sulcatus			
frondosus		×	
Uncinia † compacta			
pedicellata (endemic)			
rubra			
Carex † secta			
pumila			
Oederi			
GRAMINEAE †			
Agrostis † Dyeri			
Deyeuxia † *Billardieri*		×	
Deschampsia † Chapmanni			
Agropyrum scabrum			
Asprella gracilis			
FILICES †			
Hypolepis millefolium			
Lomaria † nigra			
Asplenium † *Lyallii*	×	×	×
Polystichum † *cystotegia*			×
adiantiforme		×	

Of these species, 21 are predicted, and 56 not, a proportion considerably smaller (largely owing to the presence of so many endemics of Stewart Island) than that given at the end of the principal table above (28 against 45 per cent.). No fewer than 14 of the 32 species given as wrongly predicted on p. 33 are included in this list, so that there only remain 18 species predicted that have not been found.

The predictions given in the paragraphs numbered 1, 2, 3, 4, 5, and 6 remain as before, with slight numerical alterations.

In prediction 7 (Stewart Island endemics) we must add nine new endemics to the ten there mentioned, as follows:

Family (in Order of Size in New Zealand).	*No. of Species in New Zealand.*[1]	*No. of Species in Stewart.*	*Endemics.*
1. Compositae	203	44	*Olearia divaricata* *Celmisia rigida*
2. Cyperaceae	117	34	*Uncinia pedicellata*
3. Scrophulariaceae	109	9	*Veronica Laingii* *Ourisia modesta*
4. Gramineae	97	30	—
5. Orchidaceae	57	21	*Azorella Cockaynei* *Aciphylla Traillii*[2]
6. Umbelliferae	54	11	—
7. Ranunculaceae	45	8	*Ranunculus Crosbyi*
8. Rubiaceae	43	13	—
9. Onagraceae	31	8	—
10. Epacridaceae	28	10	—
11. Leguminosae	25	—	—
12. Boraginaceae	24	2	—
13. Juncaceae	24	7	—
14. Cruciferae	22	3	—
15. Liliaceae	20	9	*Bulbinella Gibbsii*

[1] The new endemics must be added to these totals.

[2] Regarded above as occurring in New Zealand.

This result again emphasizes the fact that the endemics occur almost only in the very largest families (the average size of a family in New Zealand is 15 species), i. e., as we have explained, the families which are on the whole the oldest in New Zealand. If we add together the two lists of endemics, we find that 17 occur in the first seven families, and only 2 in the second eight, while the 76 smaller families contain none whatever.

The addition of these nine to the list of endemics of Stewart also confirms prediction 8, for the genera to which they belong, 9 in all, contain 249 species, or an average of 27 each, a slightly higher figure than that given for the genera containing the first ten endemics.

The other predictions given also remain as stated, with slight numerical corrections.

Nothing, therefore, in this great addition to the species found on Stewart Island compels any modification or real revision of the general thesis of this paper, and in this respect it may be compared with the revision of the figures for Ceylon which I have elsewhere published (11), and which also showed no differences in the final results.

SUMMARY.

The flora of Stewart Island is dealt with in the light of age and area as regards its taxonomic distribution. Numerous predictions are made as to what should be expected under that hypothesis, and verification is made upon the facts, all the predictions proving to be correct.

An attempt is made to predict the actual composition of the flora from what is known of the floras of the Kermadecs (1,200 miles away, at the other end of New Zealand), Chathams, and Aucklands, and 139 species of Angiosperms out of 383 are correctly predicted, and no less than 46 out of 62 ferns (74 per cent.), while only 18 species are predicted that have not been found.

Verification of the other predictions shows that—

Stewart has a greater proportionate representation of families than of genera, and of genera than of species, as compared with New Zealand.

Families are represented in proportion to the number of genera contained in them (in New Zealand).

Genera are represented in proportion to the number of species in them (in New Zealand).

Wides (the oldest forms) are best represented in the flora of Stewart.

The plants of Stewart are very widely spread in New Zealand, about twice as widely as the average.

The proportional representation of ferns is greater.

The endemics of Stewart are in the largest (in general, oldest) families of New Zealand.

The endemics of Stewart are in the larger (older) genera of New Zealand.

The Kermadec, Chatham, and Auckland Islands have proportionately more in common with Stewart than they have with New Zealand itself, although they are nearer to New Zealand than to Stewart.

All species that occur both in the Chathams and the Aucklands are also found in Stewart.

Species common to Stewart and the Chathams (84) range in New Zealand, with 4 (really 3) exceptions, up to Lake Taupo or beyond (in the North Island), i.e. to the circumference of the circle passing through Stewart and the Chathams.

The species common to Stewart and the Kermadecs (22) are most widespread in New Zealand (rarity 1·0, the minimum), those common to Stewart and the Chathams next, and those to Stewart and the Aucklands least so.

The plants common to Stewart and the Kermadecs, being the oldest, show the greatest proportion of wides, while endemic forms show in increasing proportion in the plants common to Stewart and the Chathams, or Stewart and the Aucklands.

The relationship of Stewart to the two great invasions of plants into New Zealand is then considered, and it is shown that the northern invasion was perhaps the earlier, and that Stewart did not lie in the track of the southern invasion, but received the plants thereof from the north side of Foveaux Strait. In each invasion the plants which occur in Stewart are selected from the largest families and genera.

Finally, it is pointed out that as so many predictions may be successfully based upon age and area *alone*, the operation of this factor must be the principal positive determining cause in geographical distribution, while the operation of barriers is the principal negative cause. Some difficulties which various workers have experienced in thinking of the hypothesis are also pointed out, notably that the area occupied, in the absence of barriers or other causes of limitation, increases with the square of the diameter, the latter probably increasing uniformly with the passage of time.

Bibliography.

1. Cheeseman: Manual of the New Zealand Flora. Wellington, 1906.
2. ————: On the Systematic Botany of the Islands to the South of New Zealand, in Chilton's 'Subantarctic Islands of New Zealand', vol. ii, p. 389. Wellington, 1909.
3. Kukenthal: Cyperaceae-Caricoideae, in 'Das Pflanzenreich'. Leipzig, 1909.
4. Marshall: Geology of New Zealand. Wellington, 1912.
5. Petrie: Gramina of the Subantarctic Islands of New Zealand, in Chilton. l. c., p. 472.
6. Willis: Relative Age of Endemic Species. Ann. Bot., vol. xxxi, 1917, p. 189.
7. ———: Distribution of the Plants of the Outlying Islands of New Zealand. l. c., p. 327.
8. ———: Further Evidence for Age and Area. l. c., p. 335.
9. ———: The Sources and Distribution of the New Zealand Flora. l. c., vol. xxxii, 1918, p. 339.
10. Cockayne: Report on a Botanical Survey of Stewart Island (N.Z. Dept. of Lands). Wellington, 1909.
11. Willis: The Endemic Flora of Ceylon (correction). Proc. Roy. Soc., B., vol. lxxxix, 1916.

Variation in Eranthis hyemalis, Ficaria verna, and other Members of the Ranunculaceae, with Special Reference to Trimery and the Origin of the Perianth.

BY

E. J. SALISBURY, D.Sc., F.L.S.,

Lecturer in Botany, East London College, University of London.

With twenty Figures in the Text and Tables I–X.

THE observations embodied in the present paper were made with a view to obtaining more detailed information than was available regarding the structure of 'indefinite' flowers. The results seemed sufficiently interesting to warrant their publication, since they appear to justify certain conclusions regarding the character of the perianth in the Ranunculaceae.

The material employed has been derived from several widely separated localities, in order, as far as possible, to ensure the inclusion of extremes. Reliance has, however, been chiefly placed on a detailed examination of numerous specimens from one or two habitats.

I. ERANTHIS HYEMALIS, Salisbury.

(a) *The structure of the normal flower.*

Examination of nearly four hundred specimens of *Eranthis hyemalis* shows that by far the greater number exhibit a coloured perianth of six members disposed in two whorls of three members each, thus corresponding to the one-third phyllotaxy of the foliage and scale-leaves (cf. Irmisch, 1860). As the flower emerges from the soil (Salisbury, 1916 *a*) it is protected by an involucre of three bracts. These are connate at the base and form a whorl immediately below the flower. A close examination will show that of the three bracts one is completely external, the adjacent member has one margin underlapping and the other overlapping, whilst the third is completely internal. The bracts are thus arranged in a spiral which may pass either in the clockwise or anticlockwise (Fig. 2, C) direction.

Each bract exhibits three principal lobes, which are usually deeply divided, and the whole is supplied by three vascular strands that anastomose

almost immediately on entering the leaf and again diverge, other smaller veins branching off at the same time (cf. Fig. 10, A–C).

The members of the outer whorl of the perianth are broad and, on removal, show a scar with from five to three vascular bundles (cf. Fig. 2, K), of which the three median bundles are large and the two lateral bundles (not always present) small. The three inner perianth members are narrower than the outer and usually supplied by only three vascular strands (Fig. 2, L), or less commonly by two or even one vascular bundle. In all cases there appears to be but a single strand which leaves the receptacular stele, but this sooner or later gives off two main lateral branches in succession. The variation in the number of bundles, as seen on the scar when a perianth segment is removed, is due to differences in the level at which this trifurcation takes place. Payer (1857) was therefore incorrect in writing, 'Si l'on arrache ces six pétales, on remarque dans chaque cicatrice des trois pétales externes l'empreinte de trois faisceaux fibro-vasculaires, et dans chaque cicatrice des trois pétales internes l'empreinte d'un seul faisceau fibro-vasculaire'. No distinction with regard to the vascular supply can be drawn between the members of the outer and inner whorl beyond the fact that the broader outer perianth segments usually exhibit more main strands than do the narrower segments of the inner whorl. As we shall see later, supernumerary perianth segments are by no means infrequent, and these usually are narrower even than the inner perianth segments, and, like the latter when they too are narrow, frequently exhibit a scar with only one vascular bundle. Sometimes these supernumerary perianth segments are, however, broader when the bundle branches into three almost at the level of its insertion. The vascular organization of all perianth segments is consequently essentially similar, the minor distinctions being related to their breadth.

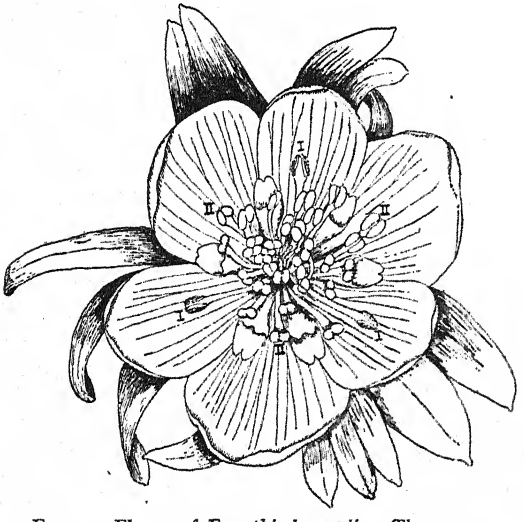


FIG. 1. Flower of *Eranthis hyemalis*. The roman numerals indicate the order of dehiscence.

Within the perianth there are most commonly found six nectaries, or honey-leaves, which arise in pairs opposite each of the outer perianth segments. The nectary on removal exhibits a scar with a single vascular

bundle (Fig. 2, M); this passes up the stalk, but on reaching the body of the nectary branches into three strands which traverse the bilobed dorsal lip.

The androecium consists of numerous stamens, the number of which, as shown in the sequel, is very variable—most commonly thirty or some other multiple of three. The stamens are generally found to form a series of radial files, the number of files being commonly double that of the nectaries. There are thus usually twelve radial series, of which six terminate peripherally in a nectary, whilst the remaining six alternate with them; the former

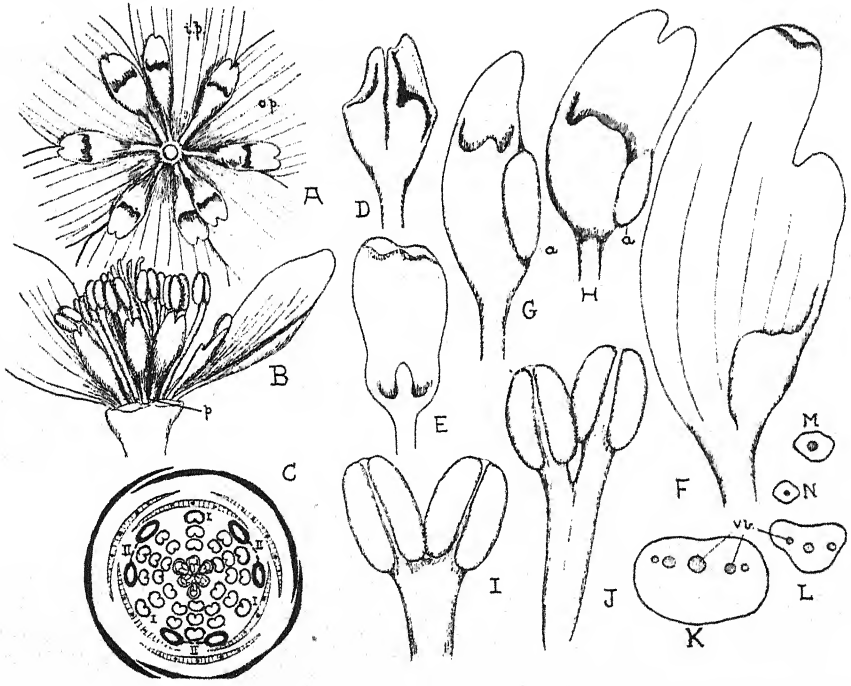


FIG. 2. *Eranthis hyemalis.* A and B, flowers with the androecium dissected away, showing the paired character of the supernumerary honey-leaves (ϕ); C, floral diagram of typical cyclic flower; D, partially bifurcated honey-leaf; E and F, petaloid honey-leaves; G and H, honey-leaves bearing anthers (a); I and J, branched stamens; K, scar of outer perianth segment; L, of inner perianth segment; M, scar of nectary; N, scar of stamen; *v.b.* vascular strand.

each consists typically of two stamens and one nectary and the latter of three stamens. To this arrangement (Fig. 2, C) there are, however, numerous exceptions (Fig. 3), the number of orthostichies being sometimes as many as twenty and not infrequently eleven, thirteen, or fourteen.

By removing a flower, before any of the stamens have dehisced, and placing it in a warm room under a low-power dissecting microscope, the whole sequence of dehiscence can be readily followed. We have confirmed the observation of Irmisch (loc. cit.), that the first stamens to mature are the three situated opposite the inner perianth segments. These are followed by

the three stamens opposite the outer perianth segments (Fig. 1). The three members of each whorl dehisce in rapid succession, followed after a shorter or longer interval by those of the next whorl within. The number of stamens, then, and their order of dehiscence, indicates that the androecium is quite commonly whorled, but a pseudo-spiral appearance is brought about by mutual pressure which results in a lateral displacement comparable to that seen in the floating rosettes of *Callitriche vernalis* or the leaves surrounding the inflorescence of *Cyperus alternifolius*. In other cases, however, the androecium is obviously spiral, frequently with a divergence of $\frac{2}{11}$ or $\frac{2}{13}$, the eleven or thirteen parastichies being clearly recognizable. As was pointed out by Irmisch (loc. cit.), the flower of *Eranthis* is then either cyclic or hemicyclic, or when, as happens but rarely, the perianth is spiral the flower may be completely acyclic.

Eichler (1878, p. 169) describes the androecium of *Eranthis* as most frequently consisting of rows of three stamens each, opposite the petals, alternating with which are six rows of four members, a total of forty-two stamens. Not only, however, have we failed to confirm this arrangement, but the number of stamens in the specimens examined seldom reached this figure.

The gynaeceum consists of a varying number of carpels, usually either five or six. In the latter case two whorls of three members each are clearly recognizable.

A drawing of a typical flower is shown in Fig. 1, the roman numerals indicating the order of dehiscence of the stamens. In Fig. 2, C, is shown by means of a diagram a typical cyclic flower.

(b) *Meristic variation.*

(1) *The perianth.* The 'curve' given in Fig. 4 shows the numerical variation exhibited by the perianth. As will be seen, the lowest number of members observed was five, but in such cases, with few exceptions, the place of the sixth member was taken in the outer whorl by a bract. The actual variation, apart from transformation, is then almost entirely in the direction of increase, the maximum number observed being nine. This unilateral character of the variation 'curve' for perianth members is frequent, and de Vries (1906, p. 740) in *Ranunculus bulbosus* has isolated a concealed secondary summit to which this asymmetry is to be attributed.

If a large number of flowers be examined one is struck by the repeated occurrence of perianth members showing all degrees of lobing, examples of which are shown in Fig. 5, E–G, and Fig. 6, C. Nearly every condition, from a mere notch to two almost completely separated halves, has been observed. Most commonly the two lobes lie in the same tangential plane, but less frequently the lobes are large and overlap to a considerable extent. When

more than six perianth segments are present the increased number of primordia may be regarded as due to a tendency to produce a polymerous perianth of which the bifurcated members are an incomplete expression.

The two antagonistic tendencies, towards trimery and multiplication of parts, have resulted on the one hand in the production of the numerous instances of supernumerary perianth segments, and on the other in the more or less complete fusions exhibited as notched and bilobed structures. That these latter are to be so interpreted seems indicated by the occasional presence of slightly notched perianth segments in which the two halves do

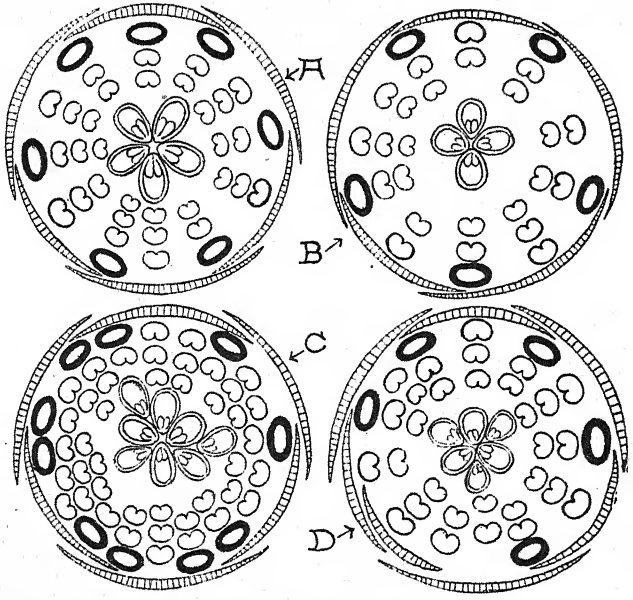


FIG. 3. *Eranthis hyemalis.* Empirical diagrams showing arrangement of honey-leaves and stamens.

not stand in the same tangential plane. As a result there is a longitudinal kink where the two halves join that seems best interpreted as due to fusion between two primordia not situated precisely side by side. The rarity of the latter phenomenon is, however, strong presumptive evidence that the fusing pairs of primordia were themselves the product of the congenital fission of a single primordium, and that subsequent fusion has taken place along the original plane of separation; a view supported by the fact that the supernumerary perianth segments are generally much narrower than those normally present (Fig. 5, 1). The position which they occupy in the flower is also in agreement with this hypothesis (cf. Fig. 6, A and C). Evidence based on numerical relations will, moreover, be adduced to show that there is no ground for the assumption that the supernumerary members are the result of transformation either of honey-leaves or of stamens. Further, the

supernumerary perianth segments often lie on the same orthostichy as the honey-leaf, hence to explain the structure of such flowers on this view a double metamorphosis must be assumed, viz. the transformation of the honey-leaf into a petal and of the next stamen within, on the same orthostichy, into a honey-leaf. Again, very rarely a supernumerary perianth segment is present and at the same time the honey-leaves are only five in

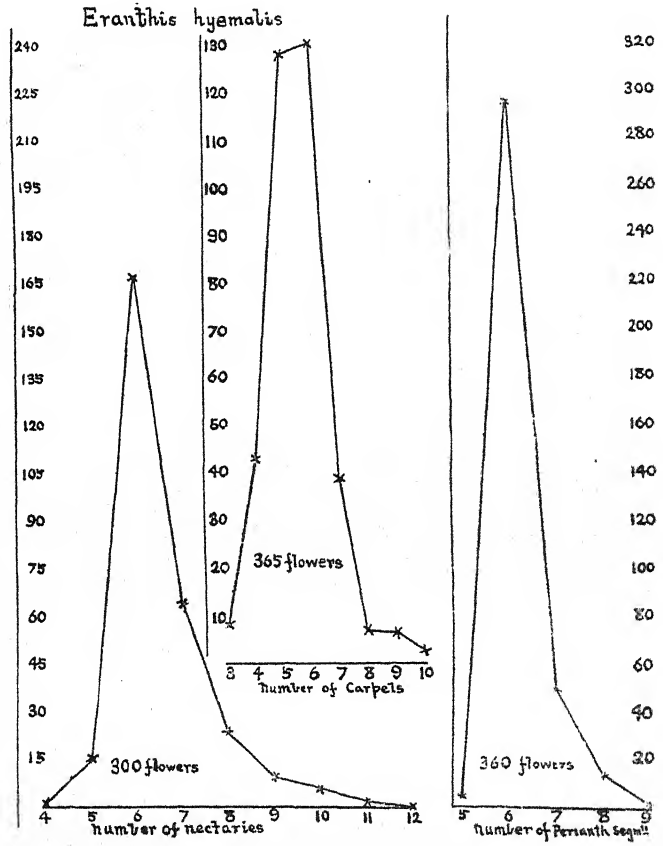


FIG. 4. Variation 'curves' for perianth, honey-leaves, and gynaeceum of *E. hyemalis*.

number, that on the same orthostichy as the extra perianth segment being absent. This might appear to indicate that, as a rare occurrence, a honey-leaf does become completely transformed, but in the case illustrated in Fig. 3, D, this explanation is not admissible, since it would involve the assumption of one more stamen on this orthostichy than are present on any one of the others. Evidently in such cases the reversion of the nectary to the staminal condition is in some way related to the space-conditions consequent upon the presence of the supernumerary perianth segment.

In any consideration of the Ranunculaceous perianth the construction which it primitively exhibited is necessarily important, and *Eranthis* sheds some light on this question. Two conditions are found in Ranunculaceae, viz. the pentamerous and the trimerous, and both are met with in the present species, though the former condition but rarely. As already stated, in most cases where there are only five coloured perianth segments this is due to the transformation of one; but rarely a true pentamerous perianth is found, and where such is the case the five members exhibit a quincuncial arrangement as in typical pentamerous species. It is clear that this condition can easily have been derived from two alternating whorls of three members each if we assume that the member which is half external, half internal, was formed by the fusion of the anterior member of the outer perianth with one

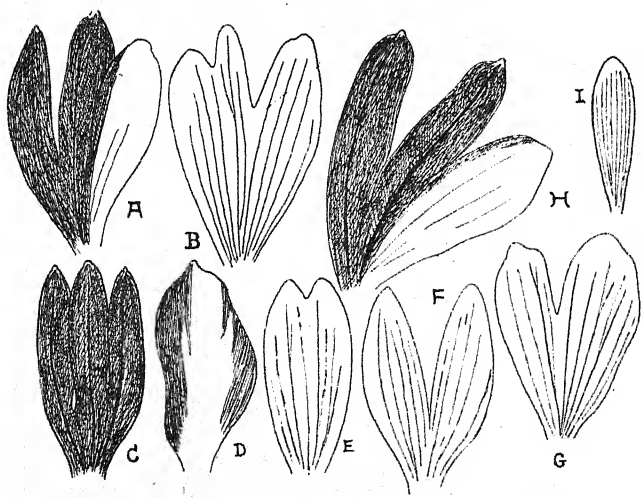


FIG. 5. *Eranthis hyemalis.* A and B, lobed perianth segments; C, trilobed bract replacing a perianth member; D, partially virescent perianth segment; E–G, lobed perianth segments; H, partially coloured bract; I, supernumerary perianth segment. Green parts indicated by shading.

or other of the lateral members of the inner perianth, according as the flower exhibits a right-handed or left-handed spiral. This would produce the typical quincuncial imbrication. That the perianth member in question has been so derived would seem to be indicated by the fact that in some cases it exhibits six principal veins in place of the usual three. The anterior perianth segment in a quincuncial flower may, moreover, be bilobed, and its attachment is broader than that of the other segments. In *Eranthis* there can be no question that the pentamerous condition has been derived from the trimerous, and in view of the widespread character of the latter type in related families it may well have been the primitive condition in the Ranales as a whole. Thus we find that in the Nymphaeaceae the genera with the least specialized type of ovary, viz. *Cabomba* and *Brasenia*, are trimerous

throughout, whilst the more specialized ovaries are associated with a multiplication of parts. Moreover, *Cabomba* and *Brasenia* exhibit a simpler anatomical structure than the other genera (Gwynne-Vaughan, 1897). A trimerous perianth is also characteristic of the families Anonaceae, Berberidaceae, Lactoridaceae, Lardizabalaceae, Menispermaceae, Magnoliaceae, and Myristicaceae, although several of these orders exhibit the usual derivative of trimery, namely, dimery. Trimery is thus associated with the arboreal habit as well as being of widespread occurrence in the Cohort.

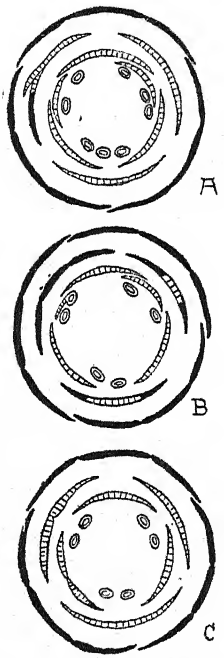


FIG. 6. *Eranthis hyemalis.* Empirical diagrams showing arrangement of bracts and perianth segments in abnormal flowers. Green parts black, coloured parts shaded. Constrictions indicate lobing.

In the Ranunculaceae both pentamerous and trimerous flowers occur in the same genus or even in the same species, though pentamery is always associated with the highly specialized zygomorphic flowers. For example, in many Paeonies there is a quincuncial calyx followed by a pentamerous coloured perianth, but in *Paeonia whitmanniana* the calyx consists of three members and the coloured perianth of two trimerous whorls (Baillon, 1862). In *Ficaria verna*, when the calyx consists of six members they occur in two alternating whorls of three members each; when the calyx consists of five members the arrangement is quincuncial (Clos, 1852). Similarly in various species of *Anemone* and *Helleborus* the perianth may consist of two trimerous, or one quincuncial whorl. In *Anemone nemorosa* both conditions occur (Yule, 1902). In *A. pulsatilla* the trimerous condition is the normal one, in *A. ranunculoides* the pentamerous. This variation is, moreover, not restricted to the Ranunculaceae, but occurs also in the Berberidaceae (cf. Eichler, 1875, Bd. I, p. 16) and is even found amongst the stereotyped Monocotyledons. *Paris quadrifolia* and some members of the Araceae, for example, not infrequently exhibit a single quincuncial whorl (cf. Smith, 1893).

(2) *The honey-leaves or nectaries.* The honey-leaves vary in number from four to twelve (cf. Figs. 3 and 6). Only one example with four nectaries was encountered, and it is worthy of note that here, as in nine out of the fifteen specimens with only five nectaries, there was no accompanying increase in the number of perianth segments. The reduced number must therefore be attributed to an actual diminution, and is probably due to replacement of one of the pairs of honey-leaves by a single member, and the orientation in such cases fully warrants this suggestion. The increase, up to as many as twelve, is doubtless the outcome of bifurcation of one or more

of the six honey-leaves usually present. In Fig. 2, A, is shown the arrangement of the honey-leaves in a flower where their number was eight, the gynaeceum and androecium having been dissected away. It will be noticed that four of the honey-leaves are grouped in pairs. The members of each pair here arise in such close proximity as to render their origin from a single rudiment highly probable. The same feature is illustrated from a specimen with seven honey-leaves in Fig. 2, B, the contiguous members being situated at the point marked *p*. In addition specimens of partially bifurcated honey-leaves are occasionally present (Fig. 2, D), the example illustrated being taken from a specimen in which there were also six normal honey-leaves. Cases such as this doubtless indicate a bifurcation of the original rudiment and the subsequent fusion of the two halves. Rarely an additional honey-

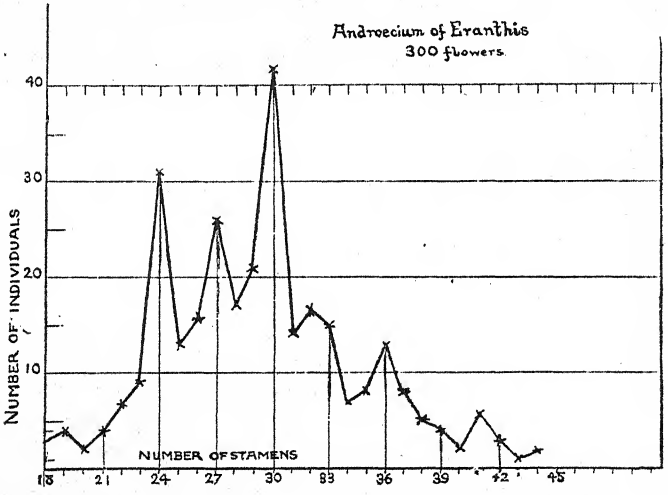


FIG. 7. *Eranthis hyemalis.* Meristic variation in androecium.

leaf is exhibited that, from its position, is to be regarded as a transformed stamen, since it is situated on the same orthostichy as one of those normally present.

(3) *The androecium.* The number of stamens ranges from 18 to 44 (cf. Fig. 7), and it is very significant that the lowest number observed should be a multiple of three. Church (1908, pp. 14–16) found that, in the specimens examined by him, the range was from 24 to 39, with 30 as the usual number. In our specimens the mode was also 30, whilst a large proportion of flowers had 24 or 27 stamens. It will be noticed that, though the general trend of the 'curve' is of the normal type, there are several maxima associated with numbers which are some multiple of three. Several instances of flowers with forked stamens have been observed (Fig. 2, I and J), and it is of importance to note that in most cases, if the forking be disre-

garded, the number of stamens is again a multiple of three. Thus in three separate flowers, in each of which one forked stamen was present, the total numbers (inclusive of the forked stamens) were 30, 33, and 36.

Having regard to the method of development of stamens and that the anther is the first part formed, it is evident that these bifurcated examples must have originated as separate papillae, though probably derived by the fission of a single primordium, the two halves of which subsequently fused. Here too, then, as in the perianth, we appear to have the two antagonistic tendencies of multiplication and maintenance of the trimerous condition. The large proportion of individual flowers with 24, 27, and 30 stamens is the outcome of the one, whilst the occurrence of numbers which are not a multiple of three is probably often the outcome of the other. Besides the examples, figured stamens have been found which were branched from the extreme base and consequently only a stage removed from two separate structures.

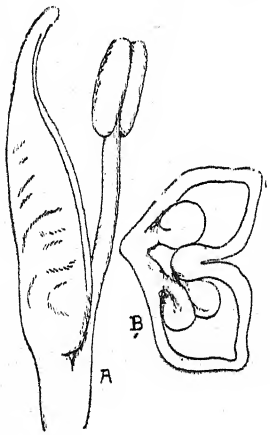


FIG. 8. Fused carpel and stamen of *Eranthis hyemalis* (A) and branched carpel of *Helleborus niger* (B).

Eranthis hyemalis is by no means alone amongst the Helleboreae in exhibiting a trimerous tendency, for, apart from the examples given later, Church (loc. cit.) states that the gynaeceum of *Helleborus foetidus* usually consists of three carpels, whilst the stamens vary in number from 30 to 54, with 45 as the normal condition. Both extremes and mean, be it noted, multiples of three.

(4) *The gynaeceum.* The carpels in the specimens examined varied from 3 to 10, though Church (loc. cit.) gives the range as from 3 to 11, and here again importance is probably to be attached to the fact that the minimum number is three. Although six carpels very frequently occur, rather more individuals were found to possess five carpels, though Church (loc. cit.) gives six as the prevailing number. The gynaeceum would thus appear to be more specialized in the direction of reduction than the androecium, a condition that is by no means uncommon in the group. Where six carpels are present, they occur in two whorls of three members each, but where the number is five they appear to be grouped in a single whorl. No case of branching or fusion of carpels has been observed here, but in the closely allied genus *Helleborus* joined carpels do occur (cf. Fig. 8, B, showing two of the carpels in *H. niger* joined together), which suggests the possibility that departures from the strictly trimerous condition may be the result of congenital fusion of carpel-rudiments. The number of ovules in each carpel ranges from six to ten, but is most commonly eight or seven (cf. Table IV).

(c) *Numerical relations.*[1]

A study of the numerical relations between the different floral regions in the same flower set forth in Tables I–IV establishes the proposition that *all the parts of the flower tend to vary at the same time and in the same direction.* Thus we find that the larger number of nectaries are associated with more numerous perianth segments, and out of a total of forty-eight flowers with more than six perianth members only five had less than the normal number of nectaries, whilst twenty-nine showed an increase. Such evidence, then, gives no support to the assumption that supernumerary perianth segments represent modified nectaries.

Still more striking is the numerical relation between carpels and stamens, or nectaries and stamens. From Tables II and III it is evident that when the number of stamens is in excess of the mean (viz. 30) the carpels and nectaries usually also exhibit an increase in number over the normal. The converse is, moreover, also true. A similar correlation is seen between the number of carpels and the number of ovules (cf. Table IV), though the number of cases examined (viz. 128) is too small to bring out this relation clearly. Hence, whatever the cause of increase or decrease, whether nutrition or some more subtle factor, it evidently tends to operate in a similar way upon the production of all the organs of the flower. The facile explanation sometimes resorted to, that increase of one type of organ takes place at the expense of another, is not borne out by the facts. On the contrary, it is clear that each type of structure is capable of exhibiting independent numerical increase or decrease without metamorphosis. Where metamorphosis does occur it is the exception rather than the rule. It is in conformity with this that the total number of parts in the flower as a whole exhibits a wide range of variation, viz. from thirty to seventy-one.

It will also be seen from the data given that in general there is a marked tendency for all the whorls of the flower to exhibit trimery at the same time.

(d) *Transition.*

We have already noted the variability in form of the bracts, and that, though usually deeply divided, they always exhibit three main lobes which may be quite entire (cf. Fig. 9). Rarely, as noted by Irmisch (loc. cit.), an additional bract may be present accompanying a normal perianth of six coloured members—doubtless the result of bifurcation. In nearly all cases, however, where there is an additional bract it is found to consist of three entire lobes and to occupy the position of one of the outer perianth segments. An instructive example is represented in the diagram (Fig. 6, B). In this

[1] The author has been unable to obtain a copy of the paper by Mori (Firenze, 1910) on the correlative variation in this species.

instance one member of the outer whorl had become transformed into a trilobed bract (Fig. 5, C) and the other two members of the whorl were undivided but exhibited a yellow central region and a green margin (Fig. 5, D). One or more members of the perianth may be trilobed and only partially virescent (Fig. 5, A). More rarely the trilobed structures are uniformly yellow (Fig. 5, B). Very rarely, too, one or more members of the involucre of bracts may exhibit partial petaloidy (Fig. 5, H). Such variations are too frequent to be regarded as mere abnormalities, for not only have they been observed in every locality from which material was obtained by the present writer, but they were recorded by Irmisch in 1860 as of frequent occurrence in the following passage: 'Nicht selten wird ein Hüllblatt mehr oder weniger vollständig in ein gelbes Kelchblatt verwandelt, oder es ist ein Kelchblatt zur Hälfte grün, zur anderen Hälfte gelb, oder einzelne Kelchblätter erscheinen, ohne die gelbe Farbe aufzugeben, nach Art der Hüllblätter mit tiefen Einschnitten, lauter Beweise für die innige Beziehung dieser Blattkreise zu einander' (1860, p. 225).

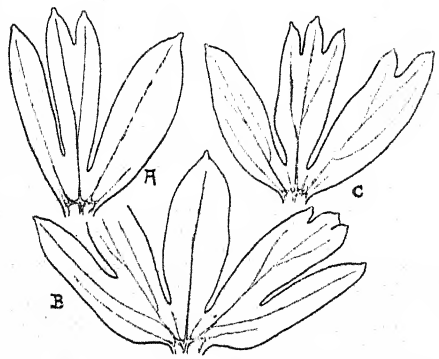


FIG. 9. *Eranthis hyemalis.* Variation in bracts.

There have thus been observed almost every possible transition between the involucral bracts and the perianth members. Moreover the latter may be replaced by the former. That such phenomena repeatedly exhibited by healthy plants have some significance can scarcely be denied, and one can therefore only conclude that either the perianth members are petaloid bracts and that both had their origin in foliar structures, or that the bracts are derivatives of the perianth members and that both had their origin from sporophylls.

That the former view is the correct one is indicated on general grounds that will be considered later, but in this particular species we may note that the replacement of perianth members by bracts is not infrequent, whilst petaloid bracts are rare, and, as is well known, atavistic variations are usually much commoner than those of a progressive character.

Moreover, the vascular supply of the perianth member corresponds closely with that of the bract, whilst even petaloid nectaries when well developed do not show the same vascular organization. It is, however, true that the supernumerary petals do help to bridge this gap.

The most important argument is undoubtedly that in no case have we found a perianth member replaced by a nectary or showing any signs of transition to one. Thus if we were to accept the other alternative we

should have the unique condition of a whorl which exhibited variations exclusively in the direction of progression.

In a few flowers nectaries have been observed one side of which bore an anther containing apparently fertile pollen (Fig. 2, G and II, *a*). These and the rare instances of two nectaries on the same staminal orthostichy show that here, as is probably the case for all the Helleboreae, the nectaries are staminodal structures. That is to say, the honey-leaves bearing anthers are to be regarded as reversions.

Not infrequently nectaries showing an enlarged petaloid anterior lobe are encountered (Fig. 2, F), thus emphasizing the potentialities of the androecium in this direction. Such petaloid nectaries are always situated on the staminal orthostichies and in this respect differ from the supernumerary perianth segments. Moreover they usually exhibit only one vascular bundle at the base and, in all the cases seen, show some indication of a nectary. An interesting example is illustrated in Fig. 2, E, where the resemblance to the staminodal petal of *Ranunculus* is striking. But this condition, whilst indicating how the adaxial scale of that genus may have arisen, is too rare to be regarded as an indication of any tendency here.

TABLE I.

Eranthis hyemalis.

Number of Nectaries.		*Number of Perianth Segments.*					*Totals.*
		5	6	7	8	9	
4	Number of specimens	—	1 100 %	—	—	—	1
5	,, ,,	1 6.6 %	9 59.9 %	4 26.6 %	1 6.6 %	—	15
6	,, ,,	—	163 92 %	12 6.7 %	2 1.1 %	—	177
7	,, ,,	1 1.5 %	50 76.5 %	6 9.2 %	7 10.7 %	1 1.5 %	65
8	,, ,,	1 4.1 %	16 66.6 %	5 20.8 %	2 8.3 %	—	24
9	,, ,,	—	4 44.4 %	3 33.3 %	2 22.2 %	—	9
10	,, ,,	—	4 66.6 %	1 16.6 %	1 16.6 %	—	6
11	,, ,,	—	—	2 100 %	—	—	2
12	,, ,,	—	1 100 %	—	—	—	1
	Totals	3 1 %	248 82.6 %	33 11 %	15 5 %	1 0.3 %	300

TABLE II.

Eranthis hyemalis.

Number of Stamens.		*Number of Nectaries.*									*Totals.*
		4	5	6	7	8	9	10	11	12	
18	Number of specimens	—	1	2	—	—	—	—	—	—	3
19	,, ,,	—	—	3	1	—	—	—	—	—	4
20	,, ,,	—	—	2	—	—	—	—	—	—	2
21	,, ,,	—	—	3	1	—	—	—	—	—	4
22	,, ,,	1	—	3	3	—	—	—	—	—	7
23	,, ,,	—	1	5	3	—	—	—	—	—	9
24	,, ,,	—	2	25	2	1	—	—	—	1	31
25	,, ,,	—	2	9	1	1	—	—	—	—	13
26	,, ,,	—	1	13	1	1	—	—	—	—	16
27	,, ,,	—	2	18	5	1	—	—	—	—	26
28	,, ,,	—	2	10	5	—	—	—	—	—	17
29	,, ,,	—	—	13	7	1	—	—	—	—	21
30	,, ,,	—	2	27	9	1	3	—	—	—	42
31	,, ,,	—	1	9	3	—	—	1	—	—	14
32	,, ,,	—	—	11	3	2	—	1	—	—	17
33	,, ,,	—	—	9	3	3	—	—	—	—	15
34	,, ,,	—	—	2	4	1	—	—	—	—	7
35	,, ,,	—	—	—	3	2	2	—	1	—	8
36	,, ,,	—	—	6	4	2	—	1	—	—	13
37	,, ,,	—	1	4	1	2	—	—	—	—	8
38	,, ,,	—	—	1	2	2	—	—	—	—	5
39	,, ,,	—	—	1	2	1	—	—	—	—	4
40	,, ,,	—	—	—	1	—	—	1	—	—	2
41	,, ,,	—	—	1	1	1	3	—	—	—	6
42	,, ,,	—	—	—	—	1	—	1	1	—	3
43	,, ,,	—	—	—	—	—	—	1	—	—	1
44	,, ,,	—	—	—	—	1	1	—	—	—	2
	Totals . .	1	15	177	65	24	9	6	2	1	300

TABLE III.

Eranthis hyemalis.

Number of Stamens.		*Number of Carpels.*								*Totals.*
		3	4	5	6	7	8	9	10	
18	Number of specimens	2	—	1	—	—	—	—	—	3
19	,, ,,	1	2	—	1	—	—	—	—	4
20	,, ,,	—	1	1	—	—	—	—	—	2
21	,, ,,	—	2	2	—	—	—	—	—	4
22	,, ,,	—	6	1	—	—	—	—	—	7
23	,, ,,	—	3	4	2	—	—	—	—	9
24	,, ,,	2	12	11	5	—	—	1	—	31
25	,, ,,	1	3	8	1	—	—	—	—	13
26	,, ,,	—	4	8	4	—	—	—	—	16
27	,, ,,	—	5	15	5	1	—	—	—	26
28	,, ,,	—	2	8	7	—	—	—	—	17
29	,, ,,	—	1	11	8	1	—	—	—	21
30	,, ,,	—	2	13	24	3	—	—	—	42
31	,, ,,	—	—	3	11	—	—	—	—	14
32	,, ,,	—	—	6	6	4	—	1	—	17
33	,, ,,	—	—	5	6	3	1	—	—	15
34	,, ,,	—	—	—	4	3	—	—	—	7
35	,, ,,	—	—	2	3	2	1	—	—	8
36	,, ,,	—	—	—	4	8	—	1	—	13
37	,, ,,	—	—	1	3	2	2	—	—	8
38	,, ,,	—	—	—	—	3	—	2	—	5
39	,, ,,	—	—	—	—	2	—	1	1	4
40	,, ,,	—	—	—	—	2	—	—	—	2
41	,, ,,	—	—	—	—	4	2	—	—	6
42	,, ,,	—	—	—	1	1	1	—	—	3
43	,, ,,	—	—	—	—	—	—	1	—	1
44	,, ,,	—	—	—	—	—	—	—	2	2
	Totals . .	6	43	100	95	39	7	7	3	300

TABLE IV.

Eranthis hyemalis.

Number of Carpels in Flower.		*Number of Ovules.*					
		6	7	8	9	10	
4	Number of specimens	—	8 66·6 %	4 33·3 %	—	—	
5	,, ,,	5 25 %	8 40 %	6 30 %	—	1 5 %	
6	,, ,,	5 11·9 %	11 26·1 %	18 42·8 %	5 11·9 %	3 7·2 %	
7	,, ,,	—	6 30 %	11 55 %	2 10 %	1 5 %	
8	,, ,,	2 12·5 %	4 25 %	4 25 %	6 36·5 %	—	
9	,, ,,	1 5·5 %	6 33·3 %	8 44·4 %	2 11 %	1 5·5 %	
	Totals . .	13	43	51	15	6	128

II. FICARIA VERNA, L.

The flower of this species (cf. Clos, 1852; Baillon, 1863, p. 4; Payer, 1857, p. 254) usually consists of a calyx of three members, exhibiting a one-third spiral, followed by eight or seven coloured perianth segments bearing nectaries. The androecium is most commonly composed of from eighteen to twenty-five stamens which show an obvious tendency to dehisce in whorls of three, though the successive whorls exhibit lateral displacement (cf. Fig. 10, G–H). The gynaeceum generally consists of from nine to twenty-four carpels, the lower numbers being often clearly arranged in trimerous whorls.

(a) *Meristic variation.*

(1) *The calyx.* The number of sepals ranges from three to seven, more than 400 of the 514 examples examined possessing the former number. The arrangement of the extra sepals as shown in Fig. 10, A–H, negatives any suggestion that these are transformed petals. It is true that the sepals are sometimes coloured, but in no instance do such bear any indication of a nectary, which is always present on virescent petals. On the other hand, the position of the supernumerary sepals (K1′–K3′) does harmonize with the view that they represent fission products of one or more of the three members normally present (K1–K3). We thus have exhibited in the calyx a phenomenon commonly met with in other members of the group and also in the other whorls of this flower. Celakovsky's dogmatic statement that these extra sepals here, and in *Anemone hepatica*, are modified members of the coloured perianth within cannot therefore be upheld. Having regard to the correlation generally exhibited between the number of parts in the different whorls, it might be regarded as strong evidence against the view

here expressed that high calyx numbers are frequently associated with a low perianth number. But the examples given (cf. Fig. 10, G and H) show that the absent petal in each case is a member of the inner whorl and not of the outer. Hence one cannot be dealing with an instance of transformation. The underlying cause is doubtless nutrition. The sepals being large in number and the first parts of the flower to develop, have entailed a drain upon the nutrition which is met by an absence of fission in the corolla, or even, as in these cases, a suppression of one member.

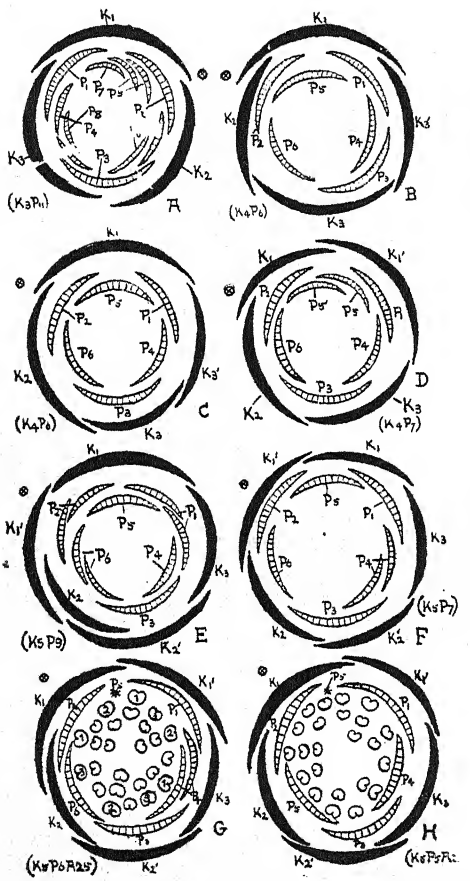


FIG. 10. *Ficaria verna.* Empirical diagrams showing arrangement of the perianth segments and stamens. The numbers indicate the order of dehiscence. K1′–K3′ indicate fission products of the calyx-segments normally present (K1–K3).

(2) *The corolla.* The number of petals ranges from four to eleven, although eight is much the most frequent. A study of the diagrams (Fig. 10) of several of the more conspicuous variants, as also of quite normal flowers, shows that the multifarious types presented are all explicable on the assumption that the corolla is typically trimerous, but that two members of the inner whorl, and sometimes all three, exhibit fission into two separate segments. The numbering of the petals in the diagrams is based on this assumption. More rarely the outer corolla whorl also exhibits fission (cf. P1, P2, Fig. 10, E). Eichler regarded these supernumerary members in both *Ficaria verna* and *Anemone hepatica* as independent additional members, but in the latter species admits the occurrence of more or less fused members (Eichler, 1875, Bd. I, p. 158). Such cases of partial fission (cf. Fig. 11, G) have been observed by the writer in several specimens, and their position fully warrants the assumption that they represent two members which in the more common condition are distinct. Very rarely additional nectary-bearing petals are present which must, from their position with regard to the staminal orthostichies and the other perianth

members, be interpreted as transformed stamens. It is important to recognize this fact that even here, where the corolla segments have obviously originated from stamens (as have the honey-leaves in other Ranunculaceae), increase in number is far more frequently an outcome of the fission of the rudiments already present than of further transformation of stamens. Hence we find a correlated increase in the androecium and corolla. In the example instanced, however, in which the position of the supernumerary petals indicated transformation, the correlated increase of the androecium was much less pronounced than the normal.

(3) *The androecium.* The variation 'curve' for this region of the flower exhibits the same salient features as in *Eranthis hyemalis*. Out of a total

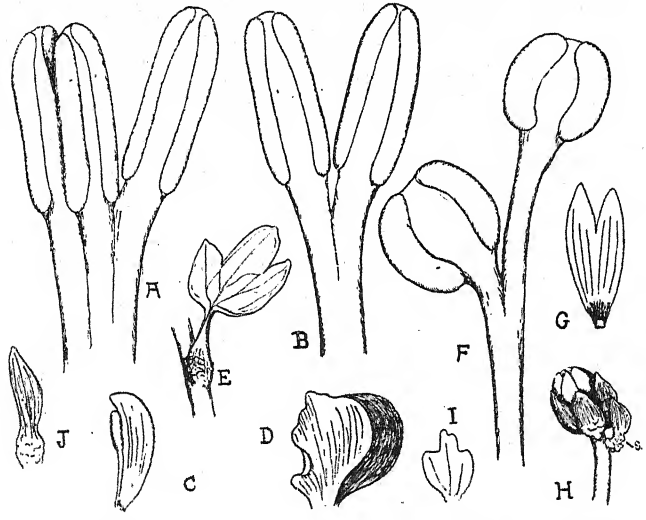


FIG. 11. A and B, branched stamens of *Ficaria verna*; C, petaloid stamen; D, partially coloured sepal; E, sepal with lobed lamina; F, *Anemone nemorosa*, branched stamen; G, bilobed petal of *Ficaria verna*; H–J, variation in the sepals of *F. verna*.

of over 500 flowers completely dissected the minimum number of stamens exhibited by any flower was a multiple of three. This minimal condition should, if the variation 'curve' were of the normal type, be only presented by a very small percentage of individuals. In actual fact there were no less than fifteen examples, a number so high as not merely to emphasize the reality of this secondary maximum, but also the abrupt character of the lower limit.

It is also very significant that the three highest numbers observed were also multiples of the three and occur as isolated points on the variation 'curve', since the largest number, not a multiple of three, was forty-nine (cf. Fig. 12).

Most frequently the androecium consists of either twenty-one or twenty-four stamens, but secondary maxima also occur at most other

multiples of three, the only notable exception being the number twenty-seven, of which there were only eleven examples; but this is doubtless correlated with the large number of flowers exhibiting twenty-eight stamens (viz. twenty-one), many of which are probably the outcome of complete fission of one of the staminal rudiments. Hence we get an increase of the higher category at the expense of the lower.

We thus have again a 'curve' which may be regarded as consisting of a number of secondary variation 'curves', each having some multiple of three as its mode. Departure from the mode is to be interpreted as an outcome

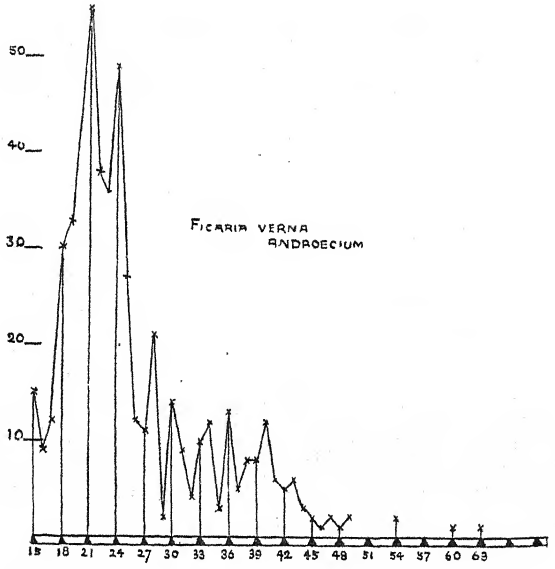


FIG. 12. *Ficaria verna*, variation 'curve' for androecium.

of fission or of fusion. Here, as in *Eranthis*, branched stamens are not infrequent, and examples are figured in which two or even three anthers occur on the same filament (Fig. 11, A and B). Rarely small abortive stamens have been observed, so that complete suppression may sometimes have taken place.

(4) *The gynaeceum.* Here again, although the feature is less marked (cf. Fig. 13), the maxima on the variation 'curve' are chiefly situated at multiples of three, the most frequent number being 15. The general trend exhibits the same markedly asymmetrical form so frequently characterizing that of the perianth.

(b) *Correlation.*

Tables V and VI bring out the fact that there is a marked correlation between the different whorls as regards either increase or decrease, so that we cannot attribute an augmented corolla to staminal transformation,

except in special cases, as that instanced above. In illustration we find that of the seven examples with eleven petals only one has twenty-four stamens, whilst the remaining six have from thirty-eight to sixty-three. Table V shows that twenty per cent. of the flowers have stamens and carpels which at the same time number some multiple of three.

(c) *Transition.*

Partially transformed stamens have been sometimes encountered, and one instance is here figured (Fig. 11, C). These emphasize the staminodal character of the petals.

By far the commonest substantive variation is that found in the calyx,

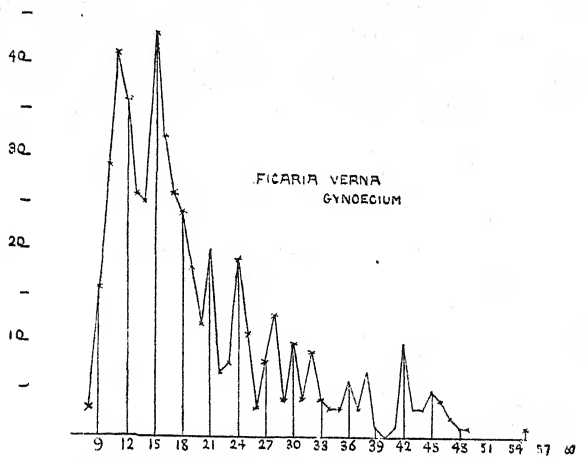


FIG. 13. *Ficaria verna*, variation 'curve' for gynaeceum.

where one or more members become more or less leafy (Fig. 11, II–J) and develop a blade that is often lobed (Fig. 11, E). There seems little doubt, from a study of all the transition stages, that the sepals really represent the basal region of modified bracts. It is also significant that where complete reversion takes place it is to the lobed, probably primitive, type of leaf, and not to the specialized simpler form of the foliage proper. Examples such as these afford the strongest evidence that the sepals had their immediate origin from foliar structures and not from sporophylls, as on the latter assumption the lobed form is inexplicable.

A partially coloured sepal is shown in Fig. 11, D, but, as has already been pointed out, such cases can invariably be distinguished from true petals. The fact that the sepals can become coloured is well exemplified in many Ranunculaceae, and merely illustrates the possibility of an attractive whorl being developed from either source of origin.

TABLE V. *Ficaria verna.* 507 flowers.

Number of Carpels in Gynaeceum.

Number of Stamens in Androecium.	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
15	–	4	1	7	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	–	4	2	2	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	–	2	–	6	–	–	1	1	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	–	2	7	1	8	–	3	3	3	–	1	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	2	2	7	2	7	5	2	3	1	–	1	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20	1	1	3	–	4	4	5	–	3	2	–	2	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21	–	–	4	11	6	2	3	10	4	7	4	2	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22	–	–	2	5	3	1	1	8	2	6	4	2	–	1	–	1	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23	–	–	–	3	4	4	2	4	6	6	–	6	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	–	–	1	1	1	6	1	9	4	3	8	–	3	3	1	1	–	3	1	–	2	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
25	–	–	–	1	1	–	5	2	2	2	2	2	–	3	–	2	1	3	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26	–	1	–	2	1	–	–	1	1	–	2	–	–	2	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
27	–	–	–	–	–	–	–	–	3	–	–	2	3	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
28	–	–	–	–	–	2	1	–	2	–	2	2	2	4	–	–	–	–	2	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
30	–	–	2	–	–	–	–	1	–	–	–	–	–	1	1	2	1	–	–	1	–	–	2	–	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
31	–	–	–	–	–	–	–	–	–	–	–	–	–	1	2	–	2	1	–	–	–	1	–	–	1	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
32	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
33	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	1	–	–	–	–	2	–	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
34	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	1	–	4	–	–	–	–	2	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
36	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	1	–	–	1	1	–	1	–	–	–	–	5	–	–	–	–	–	–	–	–
37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–	1	–	–	–	–	–	–	1	–	–	–	–	–	–	1
38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	1	–	–	–	–	–	–	1	–	1	–	–	1	1	–	–	–	–	–	–	–
39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1	–	–	–	–	2	–	1	–	–	–	–	1	2	–	–	–	–	–
40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	2	–	1	–	1	–	–	–	1	–	–	–	–	1	2	–	1	1	1	–	–	–
41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	–	–	–	–	2	–	–	–	–	–	–	1	–	–	1	–	–
42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	1	–	–	–	–	–	1	–	–	1	–	–
43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	1	1	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–
44	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–
45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
49	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–
60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	(One 52)	
63	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	(One 55)	
Totals	3	16	29	41	36	26	25	43	32	26	24	18	12	20	7	8	20	11	3	8	13	4	10	4	9	4	3	3	6	3	7	1	0	1	9	4	3	5	4	2	1	1

TABLE VI. VARIATION OF COROLLA AND ANDROECIUM IN FICARIA VERNA. 514 flowers.

Number of Stamens.		*Number of Corolla Segments.*									*Totals.*
		4	5	6	7	8	9	10	11	12	
15	Number of specimens	—	—	2	5	8	—	—	—	—	15
16	,, ,,	—	—	3	1	5	—	—	—	—	9
17	,, ,,	—	2	4	6	—	—	—	—	—	12
18	,, ,,	—	1	9	2	18	—	—	—	—	30
19	,, ,,	—	—	4	16	14	—	—	—	—	34
20	,, ,,	—	1	3	8	14	—	—	—	—	26
21	,, ,,	1	1	14	17	23	—	—	—	—	56
22	,, ,,	—	3	2	9	19	5	—	—	—	38
23	,, ,,	—	1	7	11	17	—	1	—	—	37
24	,, ,,	1	—	4	16	24	3	—	1	—	49
25	,, ,,	—	—	6	4	16	1	—	—	—	27
26	,, ,,	—	—	—	4	7	1	—	—	—	12
27	,, ,,	—	—	1	—	9	1	—	—	—	11
28	,, ,,	—	—	2	5	14	—	—	—	—	21
29	,, ,,	—	—	—	—	2	—	—	—	—	2
30	,, ,,	—	—	—	2	8	3	—	—	—	13
31	,, ,,	—	—	—	—	7	3	—	—	—	10
32	,, ,,	—	—	—	—	7	—	—	—	—	7
33	,, ,,	—	—	—	—	10	1	—	—	—	11
34	,, ,,	—	—	—	—	10	2	—	—	—	12
35	,, ,,	—	—	—	—	2	1	—	—	—	3
36	,, ,,	—	—	—	—	7	2	1	—	—	10
37	,, ,,	—	—	—	—	1	2	3	—	—	6
38	,, ,,	—	—	—	—	2	4	1	1	—	8
39	,, ,,	—	—	—	—	6	2	2	3	—	13
40	,, ,,	—	—	—	—	4	1	2	—	—	7
41	,, ,,	—	—	—	—	2	3	2	—	—	7
42	,, ,,	—	—	—	—	—	3	3	—	—	6
43	,, ,,	—	—	—	—	4	1	1	—	—	6
44	,, ,,	—	—	—	—	—	2	—	1	—	3
45	,, ,,	—	—	—	—	2	—	—	—	—	2
46	,, ,,	—	—	—	—	2	—	—	—	—	2
47	,, ,,	—	—	—	1	1	—	1	—	—	3
48	,, ,,	—	—	—	—	—	2	—	—	—	2
49	,, ,,	—	—	—	—	—	—	—	—	—	—
50	,, ,,	—	—	—	—	—	—	—	—	—	—
54	,, ,,	—	—	—	—	—	1	—	—	—	1
60	,, ,,	—	—	—	—	—	—	1	—	—	1
63	,, ,,	—	—	—	—	—	1	—	1	—	2
	Totals . .	2	9	61	107	265	45	18	7	0	514

III. ANEMONE NEMOROSA, L.

Of this species only 152 flowers were dissected, which in view of the large number of parts involved is too few to give good variation 'curves'. These, however, show clearly the tendency for maxima to occur at multiples of three (cf. Figs. 14 and 15), a feature that, owing to the small number of examples, is somewhat obscured by the overlap of the variations around the trimerous maxima. As is well known, the perianth here frequently consists of two whorls of three members each; when, however, a greater number are present the orientation of the supernumerary segments warrants the assumption that here again increase is the outcome of fission (Fig. 16, A). Where, however, the number is fewer (e.g. five) the insertion suggests fusion of one

member of the outer whorl with one of the inner, in consequence of which a quincuncial arrangement results. The involucre in one case has been observed to exhibit increase, four bracts being present in place of the usual three, so that here too we have fission exemplified.

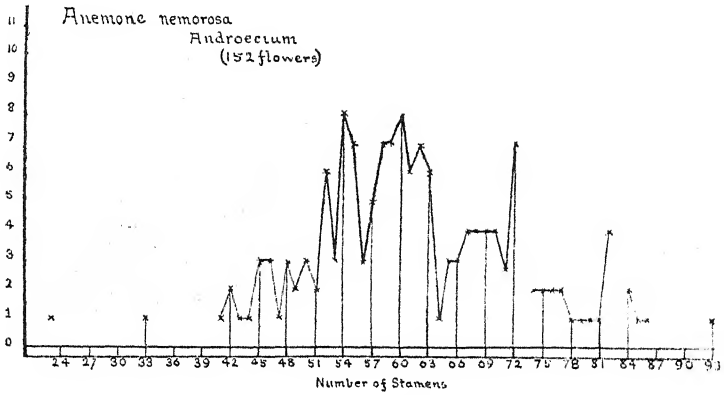


FIG. 14. *Anemone nemorosa*, variation 'curve' for androecium.

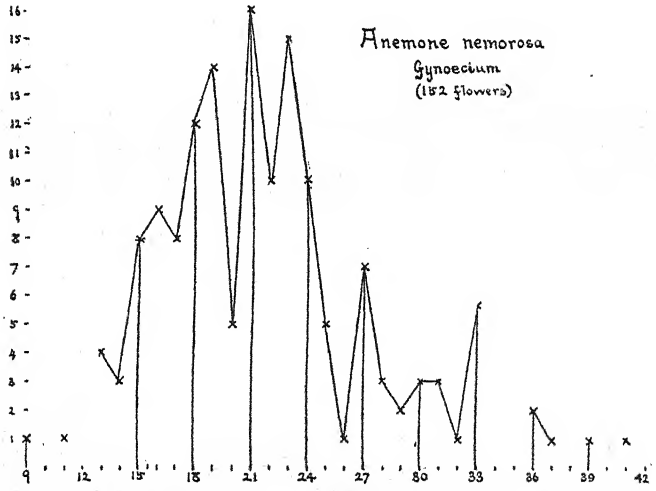


FIG. 15. *Anemone nemorosa*, variation 'curve' for gynaeceum.

As shown in Fig. 11, F, branched stamens occur, so that it would appear as if increase in the number of parts may take place in any whorl of the flower as a result of fission of rudiments already present. It will be noticed (Table VII) that, if we ignore the branching, four out of the six examples have androecia which are multiples of three.

Here too there is obvious correlation, for we find the coefficient between stamens and carpels is 0·66, whilst the probable error is 0·03.

Between perianth segments and stamens the correlation coefficient is 0·16 and the probable error 0·055. Complete or partial abortion of the male or female organs, accompanied by increase of those of the other sex, may occur in this and other species (cf. Elmore, 1915).

TABLE VII.

Anemone nemorosa.

Number of Stamens.	*Number of Branched Stamens.*	*Number of Anthers.*
54	1	55
54	1	55
54	1	55
42	2	44
59	1	60
59	2	61

Transitions are rare, but the sepals sometimes develop laminae, in which case the apical region is lobed and green, suggesting that here, as in *Ficaria*, the sepal merely represents the basal region of the bract or foliage leaf of which it is the derivative (cf. Baillon, 1863, p. 2, and Salisbury, 1916, p. 526, Fig. 1).

In eleven flowers, or about 6·5 per cent. of the total, all the floral whorls consisted of some multiple of three, the details of which are given below (Table VIII).

TABLE VIII.

Anemone nemorosa.

Perianth Segments.	*Stamens.*	*Carpels.*	*Number of Specimens.*
6	42	15	1
6	42	18	1
6	45	15	1
6	54	15	2
6	54	18	1
6	54	21	1
6	60	27	1
6	63	27	2
6	93	33	1

As there were fifty-eight flowers with stamens numbering some multiple of three, the calculated probability is that about nineteen of these should possess carpels numbering some multiple of three; in actual fact there were no less than twenty-five such cases.

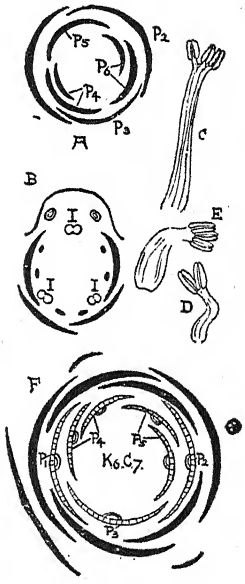


FIG. 16. A, diagram of perianth of *A. nemorosa*; B, floral diagram of *Aconitum napellus*; C, branched stamen of *Aquilegia* sp.; D and E, branched stamens of *Delphinium* sp.; F, diagram of perianth of *Ranunculus bulbosus*.

IV. Aconitum napellus, L., and A. Lycoctonum, L.

(a) *Aconitum napellus.* The fact that the gynaeceum of this species normally exhibits three carpels suggested that even here, despite the obvious specialization of the flower, the trimerous tendency might extend to the androecium.

Eighty specimens of this flower were dissected and in every case the outer perianth was composed of five members. The inner whorl, inclusive of the two nectaries, consisted of eight, or more rarely seven, six, or three members. Seventy-three flowers possessed a gynaeceum of three carpels, whilst the remaining seven had two perfect carpels. In two of these latter the third carpel was represented by a dwarfed rudiment devoid of ovules. Clearly, then, we can attribute the bicarpellary condition to the abortion of

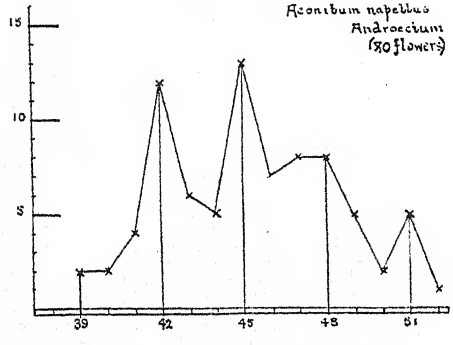


Fig. 17. *Aconitum napellus*, variation 'curve' for androecium.

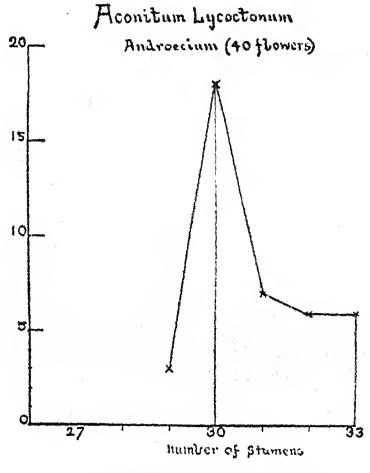


Fig. 18. Variation 'curve' for the androecium of *Aconitum Lycoctonum.*

one member, and we may note that all conditions from three to one carpel are normally encountered in the genus *Delphinium.* A study of the order of dehiscence of the stamens shows that the first to mature constitute a trimerous whorl (cf. Fig. 16, B, I, I, I), of which one member is situated opposite the hood and the two others opposite the gaps between the lateral and ventral sepals.

The variation 'curve' for the total number of stamens (Fig. 17) shows a range from thirty-nine to fifty-two, with prominent maxima at forty-two, forty-five, and fifty-one and an indication of a maximum at forty-eight. Here too, then, the minimum number is a multiple of three and the primary and secondary maxima indicate quite clearly variations around primary and secondary modes corresponding to multiples of that number. As in other Ranunculaceous flowers the actual phyllotaxis of the androecium is doubtless

but an expression of the various mechanical forces in the developing bud, and can give us no clue to the primitive construction of the flower; but the order of dehiscence and the arbitrary recurrence of certain numbers cannot be explained on purely mechanical grounds and must be construed as of phylogenetic import.

(b) *Aconitum Lycoctonum.* The androecium of this species was examined in forty unopened flower-buds obtained from wild plants in Switzerland by Prof. F. E. Fritsch. From the variation 'curve' given in Fig. 18 it will be seen that the range is narrow and that in the majority of cases the number of stamens was thirty. The lower limit observed was twenty-nine, but Eichler (1875, p. 164, Fig. 67) figures a specimen with only twenty-seven stamens. The large proportion of examples with thirty-three stamens is strongly suggestive of a second maximum. In all the flowers examined the gynaeceum consists of three carpels, so that the trimerous tendency is here even more marked than in its congener, a fact that may not be without significance in view of the greater specialization of *A. napellus.*

V. Aquilegia vulgaris, L.

We have assumed that where a frequency 'curve' shows maxima at multiples of three the explanation lies in an innate trimerous tendency, as a consequence of which the variation 'curve' is to be regarded as composed of a series of subsidiary variation 'curves' around the different multiples of three, each, however, of diminished prominence in proportion as it departs from the primary mode.

It is well known that the flower of *Aquilegia* is whorled throughout and is typically pentamerous in structure, and it was thought that the variation in the number of the carpels might afford corroboration of the above assumption by the presence of maxima at multiples of five. For this purpose 300 flowers were examined, and it was found that two maxima were present, namely, at five and ten (cf. Table IX). Even here, however, the lower limit was three carpels.

Table IX.

Aquilegia vulgaris.

Number of Carpels.	*Number of Specimens.*
3	3
4	3
5	202
6	30
7	22
8	13
9	6
10	20
11	0
12	0
13	1

As in the essentially trimerous flowers we have been considering the stamens are often not a multiple of three, so too here the number of stamens

is often not a multiple of five. Moreover, the same explanation can be advanced since partially branched stamens (cf. Fig. 16, C) are sometimes encountered and indicate the tendency towards fission of primordia.

VI. Various other Ranunculaceae.

(a) *Delphinium* sp. Fifty flowers of a species of *Delphinium* belonging to the section *Staphisagria* with three, or rarely two, carpels were dissected, and in two examples branched stamens were observed. In the one case three anthers were present on the same filament and in the second instance two (cf. Fig. 16, D and E).

The number of stamens was most frequently thirty-two, this being the case in thirteen specimens. Seven flowers had thirty stamens and eight had thirty-three. If one may judge by this small number, the mode is here not a multiple of three, but it is significant that the minimum number of stamens was twenty-seven and the number of carpels almost invariably three.

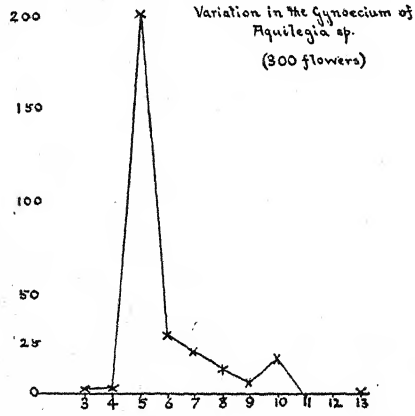


Fig. 19. Variation 'curve' for the gynaeceum of *Aquilegia* sp.

In one specimen six inner perianth segments were present, in place of the normal four, but since the androecium consisted of thirty-three stamens the increase cannot be attributed to transformation. The facts, then, seem to warrant the assumption that, here too, fission of both perianth and staminal rudiments takes place as in other members of this family.

(b) *Ranunculus bulbosus.* Out of several hundred flowers of this species forty-one exhibited supernumerary members in one or other whorl of the perianth. The details of these specimens are given in Table X. It will be noted that in only one instance was the increased number of petals associated with a diminished number of sepals, and, further, that an increase in the number of sepals was, in every case, accompanied by an increase in the number of petals. The additional sepals may therefore be regarded as the result of fission of the original sepal rudiments. The same explanation can be applied to the corolla, for we find that large numbers of petals are almost invariably associated with an increased number of stamens, as indicated by the following floral formulae of some conspicuous examples:

K 5, C 6, A 61, *G* 36
K 5, C 8, A 54, *G* 37
K 6, C 7, A 71, *G* 37
K 6, C 5, A 60, *G* 41

In addition the orientation of the supernumerary petals is quite consistent with their origin by fission (cf. Fig. 16, F, P4 and P5).

TABLE X.

Ranunculus bulbosus.

Number of Sepals.	*Number of Petals.*								
	5	6	7	8	9	10	11	12	
4	—	—	—	—	—	1	—	—	
5	—	10	7	3	4	2	—	—	
6	—	6	2	1	—	—	—	—	
7	—	1	—	1	—	—	—	—	
8	—	—	1	—	1	—	—	—	
9	—	—	—	—	—	—	—	—	
10	—	—	—	—	—	—	—	1	
Total number of specimens	—	17	10	5	5	3	0	1	= 41

A very significant feature, which emphasizes not only the distinction between calyx and corolla but also the essentially foliar character of the former, is the fact that in three out of the fourteen flowers with extra sepals, the number of peduncular ridges corresponded to the number of calyx segments. But in no case were additional ridges present when the sepals numbered five whatever the increase might be in the number of corolla segments. Coloured sepals are occasionally met with (cf. Leavitt, 1909).

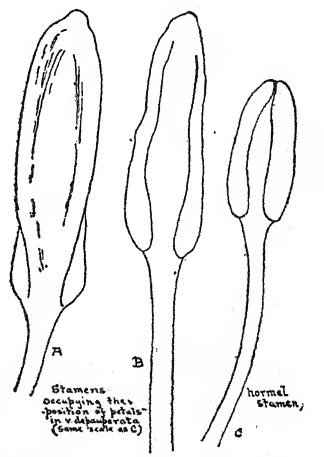


FIG. 20. Stamens of *Ranunculus auricomus*, var. *depauperata*, developed in place of the petals.

(c) *Ranunculus acris.* Seventy flowers of this species were dissected and showed a wide range of variability as regards the number of parts present. The minimum observed was seventy-nine and the maximum 275. The sepals varied in number from five to twelve, the petals from five to thirteen, the stamens from forty-four to 167, and the carpels from fourteen to eighty-three. Such extraordinary fluctuation would necessitate the examination of a far larger number of specimens before any conclusions could be drawn regarding the existence of any trimerous or pentamerous tendency. But the data are sufficient to show that each region tends to vary meristically in the same sense. In the extreme example referred to above the floral formula was K 12, C 13, A 167, *G* 83. In no instance was an increase in the number of sepals accompanied by a decrease in the number of petals. Evidence for the origin of supernumerary petals by fission is afforded by the occurrence of cleft or trilobed corolla segments. Petaloid stamens were observed in a few

specimens, and coloured sepals, though rare, were also noted. The latter, however, never show any indication of a nectary and can be readily distinguished from virescent petals. In one example a sepal was present which bore at the apex a small trilobed lamina.

(d) *Ranunculus auricomus*. This species is interesting as it affords frequent examples of reversion of petals to stamens. This condition is normally found in the so-called var. *depauperata*. In this form the position of the petals is in some specimens occupied by normal stamens, but in others by stamens which are much larger than usual or even subpetaloid in character (see Fig. 20, A–C). The instructive feature, however, is the fact that, accompanying the reversion of the corolla segments, the sepals usually become coloured yellow, whilst in the perfect-flowered form the sepals are quite green.

VII. The Origin of the Perianth.

There can be little doubt that the solitary inflorescence of *Anemone* has been derived by reduction from a many-flowered inflorescence. Thus, in *Anemone narcissiflora* two to six flowers arise together in an umbel from the involucre of bracts. In *A. ranunculoides* the number of flowers varies from three to one. In *A. palmata* the flower is normally solitary, but two flowers quite frequently occur, whilst in *A. nemorosa* the single-flowered condition has become fixed. In all these cases the flowers arise from an involucre of three or rarely four leaves which is obviously homologous in the different species. Now whilst in the case of the single-flowered members we might *a priori* imagine these bracts to represent descended perianth leaves which had become foliaceous, as Worsdell maintains (1916), such a conception is hardly applicable where we find the involucre surrounding a group of several flowers. On the other hand, the assumption that the involucre consists of foliage leaves is in complete harmony with all the facts and equally applicable to the many- or few-flowered inflorescence. Again, if we study the numerous species of *Anemone* we find almost every transition from involucral leaves in all respects resembling the foliage leaves (e.g. *A. nemorosa*), to those in which the involucral leaves are essentially calyx-like and closely approximated to the flower (e.g. *A. hepatica*). It is, moreover, significant that in general where the involucre is situated close to a flower its members depart farther from the foliaceous type than where it is more remote.

Now this transition can of course be viewed from either direction, but if the bracts forming the involucre are only modified stamens it is scarcely conceivable that their ultimate development should present all the indications of homology with, rather than analogy to, the foliage leaves. If such a striking parallel evolution had actually taken place, it could only indicate an identity of function. Yet if we examine species of *Anemone* with a simple type of leaf but in which the involucre is not in close proximity to

the flower (e.g. *A. palmata*), we find that the involucral leaves partake of the dissected, probably ancestral, foliar type and do not resemble the simple foliage of the species—a fact that cannot be explained on any other hypothesis than the common origin, and therefore phylogeny, of both bracts and foliage.

In *Ranunculus acris* Goebel (1905, p. 393) was struck by the unusual condition that the hypsophylls were more divided than the foliage leaves, and we have already noted the same feature exhibited by *Ficaria verna.*

Again, on the foliar theory we should naturally expect to find the involucre of upwardly displaced leaves more closely approximated to the flower the more specialized the floral structure. In the genus *Nigella* the different species show various degrees of fusion between the carpels; in *N. arvensis* the carpels are only slightly joined, whilst in *N. damascena* the fusion is complete. In the former the involucre is remote from the flower, in the latter closely approximated to it.

If we accept the view that this involucre is directly derived from sporophylls then we have the anomaly of the least specialized involucre associated with the most specialized gynaeceum and vice versa.

There can be little doubt that the involucres of *Anemone*, *Eranthis*, and *Nigella* are homologous structures, and there are no adequate grounds for regarding the involucre of *A. hepatica* as distinct in origin from the calyx of *Ranunculus ficaria.*

Various writers have pointed out that the Ranunculaceae present us with almost all stages in the development of petaloid stamens, from the nectariferous staminodes of *Anemone pulsatilla* to the petal of *Ranunculus.*

If we hold that the perianth is entirely staminodal (cf. de Candolle, 1823; Drude, 1887; Celakovsky, 1896 and 1900; Worsdell, 1903) then we must assume that two totally uncorrelated lines of evolution from the same type of structure are presented to us. For in *Ranunculus* or any of the Helleboreae we must assume that, first, the non-nectariferous perianth was developed from the sporophylls and that subsequently from the same origin a completely distinct type, the nectariferous petal, became elaborated. Moreover, there is no relation between the specialization of the non-nectariferous perianth and the development of the honey-leaves. In *Anemone pulsatilla* we find a highly specialized coloured perianth, together with the simplest type of nectar-secreting staminode, and in the Helleboreae the more advanced type of flower of *Helleborus* has a less specialized honey-leaf than *Eranthis.*

If we now turn to the mode of arrangement of the perianth segments we find that in the Clematideae the calyx usually consists of four members (arising as two pairs, cf. Payer, 1857, p. 252) corresponding to the four orthostichies of the decussately arranged leaves of the adult plant. In the other tribes, where the leaves are alternate and generally exhibit a one-third

or two-fifths phyllotaxis, the calyx commonly consists of from three to five members. Such correspondence between the orthostichies of the vegetative leaves and calyx-segments has no significance unless the latter be derived from the former, since the staminal orthostichies are usually much more numerous. The case of *Clematis alpina* might be regarded as an exception, since here there are four staminodal petals alternating with the four sepals. But the spacial relations here determine the position of the inner whorl just as they determine that of the corolla in *Ranunculus* or of the stamens in Rosaceae. Such an explanation cannot, however, be applied to the outermost whorl.

It is, therefore, obvious that to regard the perianth as an homologous structure derived from the stamens involves us in several assumptions for which there appears no warrant. On the other hand, these very difficulties fall into place if we accept the view that the perianth is in some cases (e. g. *Anemone*) entirely foliar in origin (cf. Prantl, 1887) and in others (e. g. *Ficaria*) derived partly by modification of leaves (the calyx) and partly from sporophylls (the corolla) (cf. Grant Allen, 1882).

Great stress has been laid by some writers on the existence in this group of polymerous perianths (Worsdell, 1916, p. 129), the argument being briefly that the supernumerary perianth segments could not have arisen *de novo* and must therefore be regarded as transformed stamens. That fission is a common phenomenon in the calyx of the Ranunculaceae, as also in the other floral whorls, has been amply demonstrated in the foregoing pages. As pointed out by de Vries (1893), the unilateral character of the Galtonian half-curves, as exhibited in the perianth variation of *Caltha palustris* or *Ranunculus bulbosus*, can be explained if we assume increase to be due to *dédoublement*. The same feature is also encountered in allied families; for example, Marchand (1863, p. 127) recorded the occurrence of *dédoublement* in the androecium of *Epimedium musschianum*, extreme examples exhibiting eight stamens in place of the usual four. The structure of the normal flower of *Podophyllum peltatum* illustrates fission both in the androecium and perianth.

Polymerous perianths can therefore be best explained as due to fission (cf. also Rendle, 1903), and the fact that increase in their members is usually accompanied by an increase rather than a decrease in the androecium renders the transformation theory in the highest degree improbable.

It has been shown that there is a fairly widespread tendency for the androecium and gynaeceum in this group to consist of parts which number some multiple of three, and examination of the variation-'curve' renders it very probable that this feature is the result of a trimerous tendency (cf. p. 64). It seems most likely, then, that an arrangement of parts on six or three orthostichies was primitive for the group, but owing to increase in the number of members through fission this arrangement has become obscured.

Such increase has resulted in mechanical pressure, which in turn has produced displacement, so that a high phyllotaxy has resulted. Perhaps, too, shortening of the torus has contributed to this effect. As already pointed out, this interpretation harmonizes with the fact that trimerous flowers are frequent amongst the Ranales as a whole and particularly associated with the less specialized and arboreal forms.

The view here put forward is, then, that the primitive Ranunculaceous flower exhibited an androecium and gynaeceum of members either arranged in alternating whorls of three members each or on six orthostichies and probably borne on an elongated axis. The essential organs were surrounded by a perianth of foliar origin also consisting of trimerous whorls. From this condition both the pentamerous calyx of *Ranunculus* and tetramerous calyx of *Clematis* have been derived, whilst the more primitive perianth of two trimerous whorls is seen in *Eranthis* and *Anemone* spp. What was perhaps the early Ranunculaceous type is seen in some species of *Magnolia* in which the whole floral structure is composed of alternating trimerous whorls.

As a subsequent specialization the outermost stamens have in some cases developed as an attractive whorl (e. g. *Ranunculus*, &c.), but in others foliar perianth members are alone present and perform both functions of protection and attraction (e. g. *Anemone pulsatilla*).

The occurrence of trimery and androecial fission as a feature of the Ranunculaceae is all the more interesting as both are found in the Alismaceae, to which Monocotyledonous family the Ranunculaceae exhibit so many resemblances.

VIII. Summary.

The variations, both meristic and substantive, in the flowers of *Eranthis hyemalis*, *Ficaria verna*, and other members of the Ranunculaceae, are described and the following facts established:

(1) Meristic variation is exhibited in all the floral regions, involving a corresponding variation in the total number of parts present.

(2) There is usually an obvious correlation between the variation in the different parts of the flower, and, in general, increase or decrease is exhibited simultaneously by the perianth, androecium, and gynaeceum.

(3) Branched stamens are not infrequently present, and bifurcated petals have been observed in several members of the group. Branched carpels are recorded for *Helleborus*.

(4) The position of the supernumerary perianth segments is consistent with their origin by fission.

(5) The two-whorled trimerous perianth of *Eranthis*, *Anemone*, &c., is sometimes replaced by a pentamerous whorl exhibiting the quincuncial arrangement, a change that can be attributed to fusion between one member of the outer whorl with one of the inner.

(6) Increase in any one region is usually accompanied by increase in the adjacent regions, so that transformation cannot be assumed.

(7) In several members of the Ranunculaceae belonging to different tribes and genera the 'curve' of meristic variation for the androecium and gynaeceum *exhibits several maxima which correspond to numbers that are some multiple of three.* The minimum number of parts present is often also a multiple of the same number.

(8) Substantive variations of the nature of transitions are not infrequent and emphasize the foliar nature of the calyx, as in *Ficaria* and *Ranunculus*, and of the entire perianth in *Eranthis*, *Anemone*, &c. Still others occur which emphasize the staminodal character of the honey-leaves.

The facts adduced appear to warrant the following theoretical conclusions:

(*a*) Meristic variation is mainly the outcome of two tendencies, viz. fission and fusion.

(*b*) The supernumerary perianth members are usually the result of fission, and only very rarely, in the case of staminodal corollas, do we find any evidence of increase as a result of transformation.

(*c*) Decrease in number is usually the result of fusion (e.g. *Anemone*, *Paeonia*), more rarely of suppression (e.g. gynaeceum of *Aconitum*).

(*d*) The perianth is either derived entirely from modified foliage leaves (e.g. *Anemone*, *Eranthis*, &c.) or in part from bracts and in part from stamens (e.g. *Ranunculus*, *Clematis alpina*, &c.).

(*e*) The flower of the Ranunculaceae is probably derived from a trimerous type, which has, however, in many cases become obscured by multiplication of parts and consequent changes in phyllotaxy, or by fusion and abortion.

Bibliography.

Allen, Grant (1882): The Colours of Flowers.

Baillon, H. (1862): Sur la fleur des Pivoines. *Adansonia*, vol. iii, pp. 45-9.

———— (1863): Mémoire sur la famille des Renonculacées. Ibid., vol. iv, pp. 1–57.

de Candolle, A. P. (1828): Théorie élémentaire de la botanique. Paris.

Celakovsky, L. (1896 and 1900): Ueber den phylogenetischen Entwickelungsgang der Blüthe und über den Ursprung der Blumenkrone. Sitzungsber. d. k. böhmischen Ges. d. Wiss.

Church, A. H. (1908): Types of Floral Mechanism. Oxford.

Clos, D. (1852): Étude organographique de la Ficaire. Ann. des Sci. Nat., Bot., 3e sér., vol. xvii, pp. 129–42.

Drude, O. (1887): Die systematische und geographische Anordnung der Phanerogamen. Schenk's Handbuch, vol. iii, pt. 2.

Eichler, A. W. (1875–8): Blüthendiagramme. Leipzig.

Elmore, C. J. (1915): Staminate Flowers in *Anemone*. Bot. Gaz.

GOEBEL, K. (1905): Organography. Vol. ii, Eng. ed., Oxford.
GWYNNE-VAUGHAN, D. T. (1897): On some Points in the Morphology and Anatomy of the Nymphaeaceae. Trans. Linn. Soc. Bot., vol. v, pp. 287–99.
IRMISCH, T. (1860): Ueber einige Ranunculaceen. III. *Eranthis hyemalis*. Bot. Zeit., pp. 221–6.
LEAVITT, R. G. (1909): Homoeosis in Plants. Bot. Gaz., p. 41, Fig. 7.
MARCHAND, L. (1863): Sur les fleurs monstrueuses d'*Epimedium*. *Adansonia*, vol. iv, p. 127.
PAYER, J. B. (1857): Traité d'Organogénie comparée de la fleur. Paris.
PRANTL, K. (1887): Beiträge zur Morphologie und Systematik der Ranunculaceae. Eng. Bot. Jahrb., vol. ix.
RENDLE, A. B. (1903): The Origin of the Perianth in Seed Plants. New Phytologist, vol. ii, pp. 66–72.
SALISBURY, E. J. (1916): Variations in *Anemone nemorosa*. Ann. of Botany, vol. xxx, pp. 525–8.
———— (1916*a*): The Emergence of the Aerial Organs in Woodland Plants. Journal of Ecology, p. 125.
SMITH, T. C. (1893): Yorkshire Naturalist. p. 246.
DE VRIES, H. (1893): Les Demi-courbes Galtoniennes. Arch. Néerlandaises, t. xxviii, pp. 442–57.
———— (1906): Species and Varieties, their Origin by Mutation. 2nd Eng. ed., Chicago.
WORSDELL, W. C. (1903): The Origin of the Perianth of Flowers. New Phytologist, vol. ii, pp. 43–8.
———— (1906): Principles of Teratology. Vol. ii, Ray Society.
YULE, G. U. (1902): Variation in the Number of Sepals in *Anemone nemorosa*. Biometrika.

ERRATUM

No. CXXVII. Page 371, line 27
For 'tip of the primary rachis' *read* 'tip of the secondary rachis'

On an Australian Specimen of Clepsydropsis.

BY

B. SAHNI, M.A. (Cantab.), M.Sc. (Lond.).

With Plate IV and two Figures in the Text.

I. Introduction.

THE specimen forming the subject of this paper is from the private collection of Mr. A. B. Walkom, of the University of Brisbane. It was collected near Mt. Tangorin, New South Wales (long. 151° 22′ E., lat. 32° 36′ S.), and, although not discovered *in situ*, is, according to the owner, probably of Carboniferous age. More definite information regarding the horizon is unfortunately lacking.

A cursory examination of the sections showed that the fossil should be referred to the Zygopterideae, and that on account of the shape of the petiolar trace (as seen in transverse section) it would have to be placed near *Clepsydropsis antiqua*, Unger. This fact had an additional interest at the time, for I was then under the impression that this was the first specimen of a Zygopterid to be recorded from Australia—in fact from any other part of the world except Europe and one locality in Western Siberia. The work was therefore carried on with increased interest: the greater part of it was completed early in the year 1917, and a preliminary account was read before the Cambridge Philosophical Society on February 19, 1917. Only a few days before that date, however, I received from Professor W. H. Lang a letter for which I am much indebted to him. In that letter he informed me that Mrs. E. M. Osborn, of Adelaide, Australia, was also investigating a Zygopterid from Australia, and referred me to her preliminary account of it in the Annual Report of the last Manchester meeting of the British Association (1915),[1] of which I was unfortunately not aware. Mrs. Osborn's fossil also comes from New South Wales, but from a locality near Barraba, about 150 miles north-west of Mt. Tangorin, and was discovered *in situ* in rocks probably of Upper Devonian age. From the short sketch just referred to it was obvious that the two plants belonged to the same genus, but it was impossible to say whether the Barraba fossil was specifically distinct from

[1] pp. 727–8.

[Annals of Botany, Vol. XXXIII. No. CXXIX. January, 1919.]

the Mt. Tangorin fossil. On the advice of Professor Seward a brief description of the Mt. Tangorin specimen, accompanied by a few rough figures, was sent to Mrs. Osborn for comparison; in her answer (received in September 1917) she states that there is no doubt as to the specific identity of the two, and proposes the name *Ankyropteris australis* (see, however, pp. 83–4, below). Two valuable features about the Barraba fossil are, firstly, that the stem is preserved, and, secondly, that the origin of the leaf-trace is visible. The Mt. Tangorin specimen, on the other hand, which does not include the stem, and is on the whole not well preserved, contains portions of the petioles slightly more distal than those in the Barraba fossil, and in the further branching of the petiolar trace to a very small extent appears to supplement the structures preserved in the latter.

I am very grateful to Mrs. Osborn for the great kindness she has shown me in comparing my description and in so willingly supplying me with unpublished details about her own fossil, which have been of much help and interest. It is also my pleasant duty to express my heart-felt thanks to Professor Seward, who was kind enough to entrust the work to me, and who has been a constant source of help and encouragement. The work was carried out during the tenure of a Research Studentship at Emmanuel College, Cambridge, and latterly also of a Grant from the Dixon Fund of the University of London.

II. Review of Previous Literature.

The name *Clepsydropsis*, which has reference to the cross-section of the petiolar trace, was first used by Unger in 1854,[1] but it was not until two years later[2] that he published a diagnosis of the genus, placing it in Corda's[3] family Rachiopterideae, and described (provisionally as three distinct species, *C. antiqua*, *robusta*, *composita*) some fern-petioles from the Cyprid Schists in the Upper Devonian (Lower Culm) of Saalfeld in Thuringia. Unger's originals have since been several times re-examined, and from Solms-Laubach's[4] work (1896), as well as from a more recent paper[5] by Dr. Paul Bertrand, it appears that the two last-named species were founded upon deformed specimens of *C. antiqua*, Ung. In 1889 Stenzel[6] described another species, *Asterochlaena* (*Clepsydropsis*) *kirgisica*, Stenz., from the vicinity of Pawlodar, north of Semipalatinsk in Western Siberia, about the horizon of which we have no certain data. The fossil was discovered as a pebble in an alluvium overlying coal-bearing strata, and was considered to be of Lower Permian (Rothliegende) age,[7] but this is by no means free from doubt.[8] The original of Stenzel's Fig. 38, Pl. IV, collected by Åberg

[1] Unger (1854), p. 599.
[2] Unger (1856), p. 165.
[3] Corda (1845).
[4] Solms (1896), pp. 25–7.
[5] Bertrand (1911 *a*), p. 4.
[6] Stenzel (1889).
[7] Stenzel (1889), p. 20.
[8] Solms (1910), p. 542. See also Goeppert u. Stenzel (1881), p. 16.

and conveyed to Germany by Ludwig, was at Dresden, but Stenzel also examined a slice in the possession of Goeppert.[1]

Hitherto two species, namely, *C. antiqua*, Ung., from the Upper Devonian of Thuringia, and *C. kirgisica*, Stenz., *doubtfully* from the Lower Permian of West Siberia, have been recognized. The published descriptions do not reveal any differences that are clearly of specific value, but, pending a more detailed examination of Stenzel's fossil, it is prudent to keep the two species apart.

The most recent paper directly bearing upon the subject is Mrs. Osborn's 'Preliminary Observations on an Australian *Zygopteris*',[2] which is of great interest as being the first record of the Zygopterideae from outside of Europe and Siberia. In this paper two important points, on which there has been a good deal of speculation in the past,[3] have been definitely settled. Firstly it has been shown that the cauline xylem cylinder is of the *Ankyropteris Grayi* type, and secondly that the leaf-trace is nipped off as a closed ring of xylem enclosing a portion of the 'mixed pith' of the stellate axial cylinder, and that this ring subsequently becomes flattened, with a slight curve convex on the adaxial side (as is often the case in *Ankyropteris*). The curve finally disappears, and a slight median constriction imparts to the trace the form well known for the genus *Clepsydropsis* (see Text-fig. 2, *1*). There are no axillary branches.

III. Nomenclature.

As already mentioned, Mrs. Osborn has proposed to place the fossil in the genus *Ankyropteris*, a perfectly natural course, considering the stem structure and the mode of origin of the leaf-trace. On the other hand, the shape finally assumed by the leaf-trace would by itself lead to a reference of the plant to the genus *Clepsydropsis*. In fact the latter course was at first actually adopted in the case of the Mt. Tangorin specimen, described below, in which the stem and the leaf-trace origin are not preserved. However, the combination, in the Barraba specimen, of the structural

[1] In the same year Schenk (1889, pp. 553–4; see also Schenk, 1890, pp. 46, 156) figured and briefly described a fossil (*Rachiopteris Ludwigii*, Leuckart u. Schenk) mainly agreeing with Stenzel's description of *C. kirgisica*. The original of Schenk's outline drawings (Pl. III, Figs. 50, 51) was in the Botanical Collection at Leipzig and was named by him after Ludwig, who collected it in the Ural steppes between Akolinsk and Semipalatinsk, and Leuckart, through whose agency it reached Leipzig. The ultimate sources of the two originals are so nearly identical as to leave little doubt that they were portions of the same specimen, yet neither Stenzel nor Schenk refers to the other's paper; on the contrary, both the authors seem to imply that their respective fossils were till then undescribed. There is on this point a regrettable confusion in the literature, which, in spite of Solms-Laubach's (1910, p. 543) attempt to explain it, appears not yet to have been removed. Only *C. kirgisica*, Stenz., has been recognized by subsequent writers, although it is possible that *C.* (*Rachiopt.*) *Ludwigii*, Leuck. u. Schenk, may be distinct, for Schenk's figures of the transverse sections show the petiolar trace in this fossil at least half as long as the petiolar diameter.

[2] Osborn (1915), pp. 727–8.

[3] Bertrand (1908, 1911 *b*, 1911 *c*, 1912, 1914); Solms (1910), pp. 540–1.

features of both *Clepsydropsis* and *Ankyropteris* strongly suggests that these two genera should be united. This step was proposed in a recent paper,[1] and in the following pages the genus *Clepsydropsis* will be understood to include *Ankyropteris*.

It is of some interest to find that this conclusion is fully corroborated by some photographs of *C. antiqua*, Ung., published by Dr. P. Bertrand in 1911 (1911 *a*, Pl. II, Fig. 16; Pl. I, Figs. 1, 2+3 and 4+5; also Bertrand, 1912, Fig. 21, p. 228). These figures show beyond doubt that the mode of leaf-trace origin in that species was essentially the same as that in the Barraba fossil, and that known for *C.* (*Ank.*) *Grayi*, Will., if we disregard the complications due to the presence of the axillary branch. (See Fig. 35, p. 244, in *Progressus*, 1912.)

The figures in question are based upon a single specimen, discovered by Dr. Bertrand among Unger's originals. The unusually small dimensions of the petiolar strand led him to conclude that it was probably either an abnormally thin petiole or a normal petiole sectioned in its distal region. Dr. Bertrand, however, evidently overlooked one rather important point in the figure, which shows that it undoubtedly represents a section across the *basal* region of a petiole. This is the presence, in the tangential plane, of a plate of narrow tracheides forming a bridge between the two peripheral loops and similar to the tracheides lining the loops themselves. The significance of this feature at once becomes apparent when we consider that the clepsydroid shape of the strand in the Barraba fossil is the result of the tangential flattening, followed by a median constriction, of a closed xylem ring lined internally by a zone of specially narrow tracheides. An exactly similar condition was observed in one of the petiolar strands in the Mt. Tangorin specimen, and is described below (p. 88; see Fig. 6, Pl. IV). Subsequently I learned from Mrs. Osborn that the same feature is visible in her specimen, and that it persists for a short distance from the leaf-base upwards.

A diagnosis of the genus *Clepsydropsis* as extended in accordance with the above suggestion is given in my paper above referred to, along with other theoretical considerations.

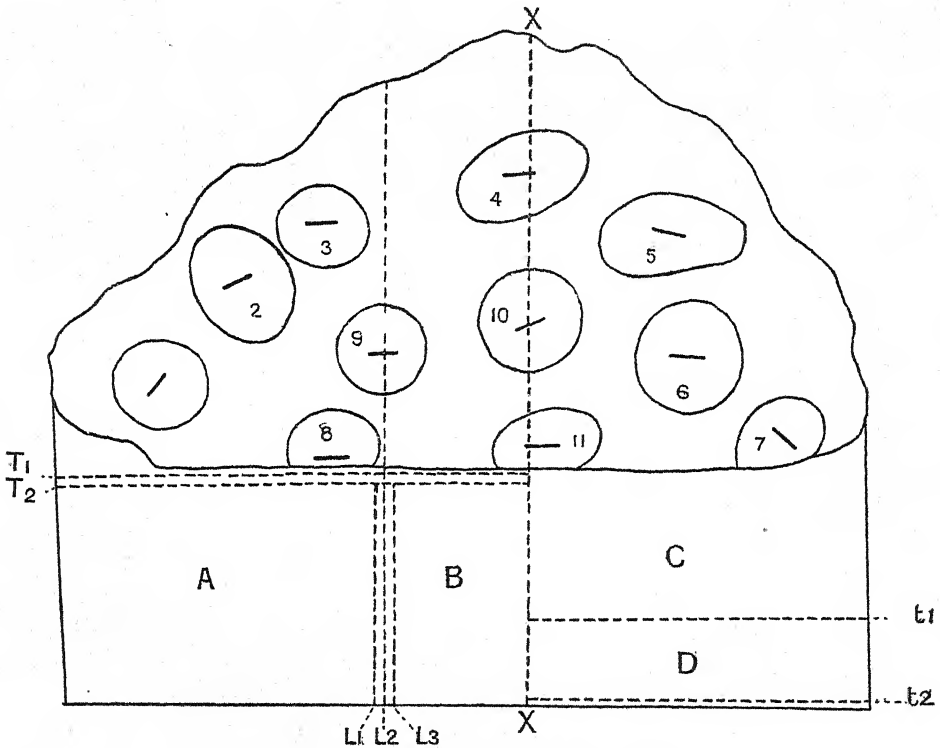
IV. Description.

Gross features. When received the fossil was an almost semicircular slab about 3·5 cm. thick, with the diameter of the semicircle about 11 cm. long. The more or less flat upper and lower faces showed the cut ends of about a dozen cylindrical petioles varying in diameter from about 15 to 17 mm., a few of which were evidently compressed into unnatural shapes. The spaces between the petioles were packed with numerous occasionally branched roots running in all directions. These were possibly mixed with

[1] Sahni (1918).

pinnae and aphlebiae, though these could not definitely be made out. On the polished faces the characteristic petiolar strands could be observed in several of the leaves, and when the surface was covered with a thin layer of oil, with the help of a lens the course of the roots and petiolar strands could be traced for some distance into the transparent siliceous matrix.

Text-fig. 1 is a diagram to show the fossil in plan and elevation; the outlines of the plan were drawn from the lower end of the fossil. The block was first cut into two pieces along XX, and from the left-hand piece



TEXT-FIG. 1. Diagram. For explanation, see text.

the two transverse sections T 1, T 2, and the three longitudinal sections L 1, L 2, L 3 were prepared. Subsequently two transverse sections *t* 1, *t* 2 were prepared from the right-hand piece. The original specimen is therefore now in four pieces, A, B, C, D, besides a fragment left over after levelling one of the ends. In the figure the petioles are marked with numbers to correspond with those in Text-fig. 2.

The orientation of the petiolar strands (indicated by short straight lines in Text-fig. 1) shows that the leaves are arranged in their natural positions round a stem which is, however, missing. The longitudinal sections show the petioles sloping inwards towards the stem side, but from the low angle

at which they stand to the vertical it may be concluded that the leaves were probably almost erect in their basal region. These facts make it very probable that the plant was a fair-sized tree-fern resembling the fossil Osmundaceae.

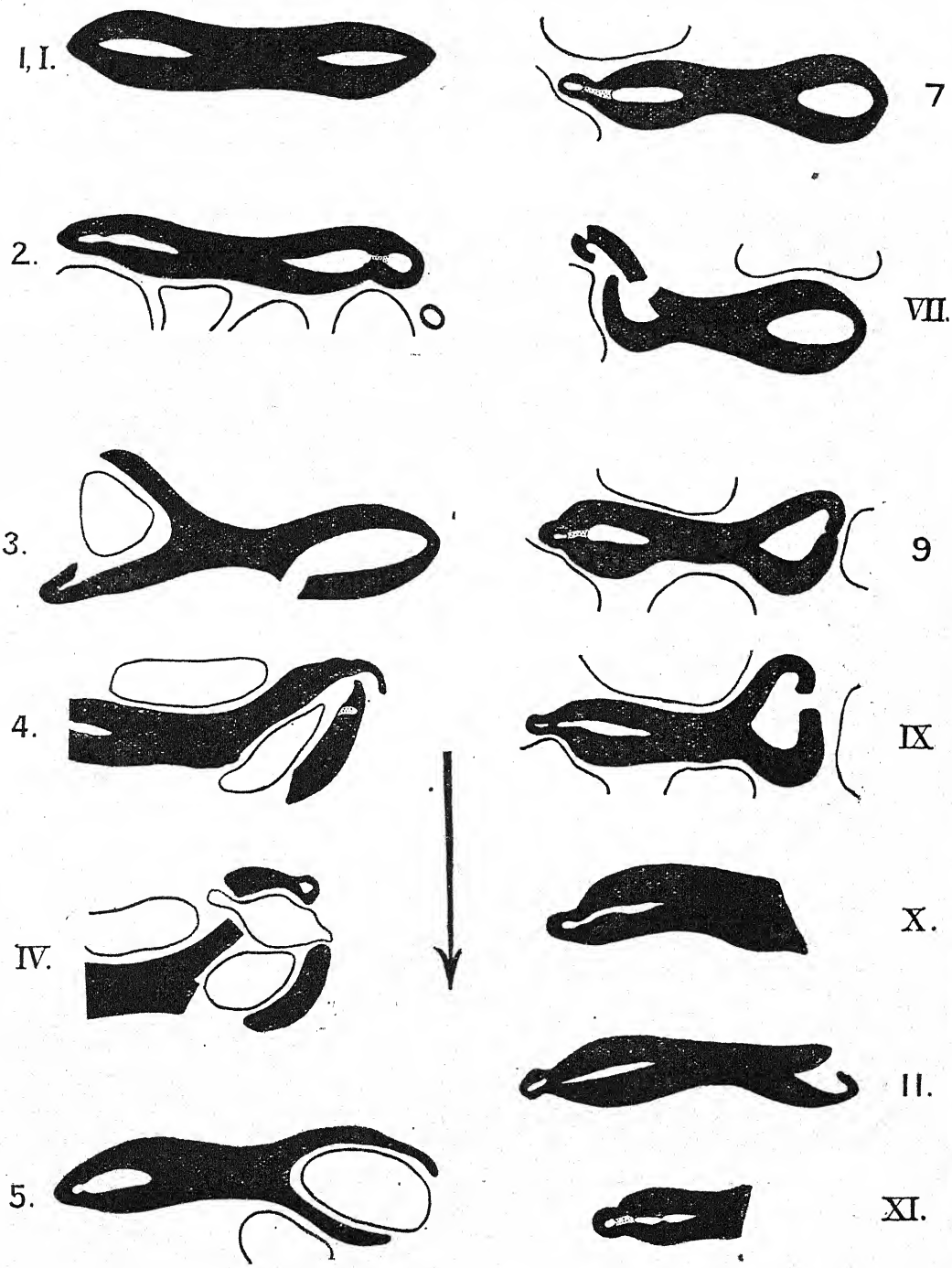
Anatomy. The preservation on the whole cannot be described as good, although some portions show well-preserved details of structure. Many of the roots have penetrated deep into the cortex of the leaf-stalks, and have even distorted the shapes of most of the petiolar strands.

The *roots* probably all belong to the *Clepsydropsis* itself, although none of them is seen actually connected with the leaves—they may be arising from the more proximal regions of the latter and in part even directly from the stem, as appears to be the case in the Barraba specimen, and as Stenzel (1889) describes in *C. kirgisica* (p. 22, Pl. IV, Fig. 38, *w'*). They have a diarch xylem strand (Pl. IV, Fig. 1) and the pitting of the metaxylem tracheides was finely scalariform as in the leaf-strand. The *cortex* of the petiole consists of a broad inner zone of large thin-walled cells with intercellular spaces, succeeded on the outside by a narrower zone of cells gradually decreasing in diameter towards the epidermis (Pl. IV, Fig. 2). In longitudinal section the cells of the inner cortex are nearly isodiametric, those of the outer cortex more elongated, with transverse or oblique end-walls (Pl. IV, Fig. 3). The *epidermis* is not preserved, consequently the 'external glands or hairs' mentioned by Mrs. Osborn are not seen. A few of the outermost layers of the cortex appear to be specialized as a thickened *hypodermis*, as described by Schenk in *Rachiopteris Ludwigii* (1889, p. 554), but it is difficult to be clear on this point. The total radial thickness of the preserved cortex varies from 3 to 4 mm.; between it and the petiolar strand the leaf tissues are nowhere preserved. In Schenk's fossil just referred to this space was in part at least (*loc. cit.*, p. 553) occupied by a sclerotic sheath.

The adjoining figures, from camera-lucida sketches of the petiolar strands, illustrate the extent to which the intruded roots (shown in rough outline only to indicate their positions) distort the normal clepsydroid shape of the leaf-traces. Only one of these happens to be completely undisturbed (Text-fig. 2, *1*, 1). It was seen cut at two different levels (T 1, T 2) and its dimensions are given below:

Total length, 6·4 mm.
Thickness at the 'waist', 1 mm.
Length of each peripheral loop, 1·7 mm.
Distance between the inner ends of the two loops, 2·1 mm.

The variations in size could be judged from the remaining figures, but the amount of distortion makes it unsafe to base any measurements upon them. The limits of the xylem could not in all cases be accurately observed, owing to imperfect preservation. In many places only rows of dirt-granules are seen, which, however, unmistakably marked the contours



TEXT-FIG. 2. The petiolar strands, which are all shown with their adaxial faces in the direction of the arrow, are marked with numbers to correspond with those in Text-fig. 1. The Roman numerals refer to the petioles as seen in the transverse sections T 2, *t* 2; the corresponding Arabic numerals to the respective petioles, as seen in the transverse sections T 1, *t* 1. For further explanation, see text, p. 86.

of the tracheides. Only those parts are shown in solid black where outlines of tracheides were recognized with tolerable certainty. The dotted areas refer to parts where the existence of xylem was only inferred.

The shape of the trace at once decides the specific distinctness of the plant from either *C. antiqua*, Ung., or *C. kirgisica*, Stenz. It is proportionately thinner, has pointed instead of truncated ends, and the internal contour of the peripheral loops is fusiform instead of elliptical.

The tissue filling the peripheral loops, which in the Barraba fossil consists of parenchyma intermixed with small tracheides, is not preserved at all. The loops are instead filled up by a deposit of mineral granules in the form of peculiar branched tubular structures, the appearance of which suggests that they were probably formed in the same manner as a continually growing semi-permeable membrane round a crystal of copper sulphate in a solution of potassium ferrocyanide. A similar process was probably responsible for a pseudo-cellular structure replacing the cortical cells in some of the leaves, each pseudo-cell having been formed round an independent centre.

The tracheides are practically all scalariform (Pl. IV, Fig. 4), with occasional anastomoses between the bars of thickening, as an approach to the reticulate type. The peripheral loops are lined with tracheides distinctly narrower than those immediately outside them. As we pass still farther away from the loops the tracheides again diminish in size (Pl. IV, Fig. 5). Only one spiral element was observed; of the others some on account of their small diameter gave the impression of being annular. In a few scalariform tracheides the pits, which were rather farther apart than usual, showed in surface view what may be called 'false borders', because in sectional views they could not be seen at all. It may be that the free edges of the thickening bars, instead of hanging over the pit so as to narrow the entrance to it, sloped in the opposite direction, so that the entrance to the pit became wider than the bottom. The effect in surface view would in either case be almost the same. The tracheides average about 100 μ in diameter; the largest seen was 180 μ, the smallest 20 μ across.

In one of the leaf-traces the inner ends of the two peripheral loops were connected by a series of narrower tracheides similar in size and form of pitting to those lining the loops themselves (Pl. IV, Figs. 6, 7). The significance of this feature has already been explained on p. 84.

Dr. Bertrand (1911 *c*, p. 509) records the presence in the leaf-strand of *C. antiqua* of a number of tracheides of secondary origin. No trace of secondary xylem was present in any of the leaf-strands of the Mt. Tangorin fossil.

The origin of the pinna-trace is exactly as in *C. antiqua*, that is, the trace is constricted off as a closed ring from the end of the peripheral loop. Unfortunately the large number of intrusions have greatly disturbed the

relative positions and shapes of the different parts of the xylem, and in a few cases (Text-fig. 2) they have even broken the latter into two or more pieces.

One of the leaves shows further branching of the vascular supply (Text-fig. 2, *2*). In this case a small elliptical ring of xylem is seen lying just off the pinna-trace, adaxially to it, at an angle of 45°. Owing to bad preservation, in the only other section that passes through this leaf there is no sign of this strand, so that it is at present impossible to ascertain its point of origin, whether from the pinna-trace or directly from the primary leaf-strand; but from the direction of the long axis of the ellipse (due to oblique section of a circular tube) it seems highly probable that it arose from the pinna-trace. In view of the proximity of intruded roots, however, it is quite likely that the strand in question has been shifted slightly out of its natural position. The obliquely-cut tracheides show a finely scalariform pitting; no spiral or annular elements are seen. The thickness of the ring is about three tracheides; the tissue in the centre of the ring is not preserved.

V. Theoretical.

Aphlebiae. In *C. antiqua* Dr. Bertrand (1911 *a*, pp. 15–17) has figured a small ring-like strand occupying a rather similar position. This strand evidently has the same morphological value in both cases. Dr. Bertrand regards it as the trace of a pinnule (tertiary rachis). It is probable, as Professor Seward suggested, that the organs supplied by these tertiary strands were of the nature of aphlebiae. This supposition is confirmed by a comparison with the corresponding structures in *Diplolabis Roemeri*, *Metaclepsydropsis duplex* (Gordon, 1911 *a*, *b*), *Stauropteris oldhamia* (Bertrand, 1909, p. 29, Fig. 4). The strands referred to by Stenzel (1889, p. 45) as arising from the base of the leaf-trace are also probably of the same nature.

Better preserved material is necessary to decide the question as to the normal number and position of the aphlebia-strands on each side of the pinna in *Clepsydropsis* (Unger, 1856, p. 167; Solms, 1896, pp. 25–7; Stenzel, 1889, pp. 21, 23); a re-examination of Stenzel's original specimens of *C. kirgisica* would be useful.

Habit.—The known genera of Zygopterideae may be classified into two groups, the Clepsydroideae (including the genera *Asterochlaena* and *Clepsydropsis*) and the Dineuroideae (including *Dineuron*, *Diplolabis*, *Metaclepsydropsis*, *Etapteris*, *Stauropteris*, *Zygopteris*, and provisionally *Gyropteris sinuosa*, which may ultimately have to be placed as a species of either *Diplolabis* or *Metaclepsydropsis*). Except for *Gyropteris*, these two groups are co-extensive with those proposed by Kidston and Gwynne-Vaughan (1910, p. 470) on the basis of the number of (free) branch-axes on each side

of the primary rachis. It is interesting to find that, so far as our present knowledge goes, the symmetry of the stem in the two groups is quite distinct. Thus in the only genera of the Dineuroideae in which the stem is known, viz. *Diplolabis* and *Metaclepsydropsis* (Gordon, 1911 *a*, *b*), it had the form of a creeping rhizome with the leaves confined to the dorsal side and rising perpendicularly from it. On the other hand, in the Clepsydroideae, in which our knowledge of the stem is more complete, the leaves arose radially on all sides of the stem, which was either an upright stock (*Asterochlaena* (Bertrand, 1911 *b*), *C. kirgisica* (Stenzel, 1889, Fig. 38, Pl. IV), and the Australian *Clepsydropsis*) or, as in *C.* (*Ank.*) *scandens* (Stenzel, 1889; Scott, 1909), it scrambled among the roots on the upright stem of a *Psaronius*.

It appears as if the precocious bifurcation of the pinna-trace in the Dineuroideae (except *Gyropteris*), and the consequent peculiar habit of the petiole in that sub-family, had something to do with the manner in which the basal portion of the leaf was held. One is tempted to expect that the stem of the remaining Dineuroideae will also be a horizontally creeping rhizome.

VI. Summary.

A description is here given of a *Clepsydropsis*, collected near Mt. Tangorin, New South Wales, in rocks possibly of Carboniferous age. It is specifically identical with the fossil briefly described by Mrs. Osborn in the Report of the British Association (Manchester meeting, 1915, p. 727).

It is shown that in *C. antiqua*, Unger, also, the leaf-trace arose as a closed ring of xylem which became tangentially flattened, and then became clepsydroid as the result of a median constriction. This fact strongly supports the suggestion put forward in an earlier paper that the genera *Clepsydropsis* and *Ankyropteris* should be united, the leaf-trace origin in the latter genus being known to be essentially the same.

The two groups of the Zygopterideae (Clepsydroideae and Dineuroideae) were also sharply distinct in the symmetry of their stem, which, so far as we know, was radial in the formal group and dorsiventral in the latter. It is suggested that this distinction will be maintained as our knowledge of the stem becomes more complete. The apparently radial symmetry of the basal region of the leaf in the Dineuroideae (due, as previously shown, to an early bifurcation of the pinna-trace) may also be related to its probably strictly upright position in that sub-family. The opinion has already been expressed in an earlier paper that this apparently radial symmetry was not continued into the probably more or less horizontal laminated distal portion of the leaf.

Botany School, Cambridge,
April 1, 1918.

BIBLIOGRAPHY.

BERTRAND, PAUL (1908): Sur les stipes des *Clepsydropsis*. Comptes Rendus, cxlvii, pp. 945–7.

——— (1909): Études sur la fronde des Zygoptéridées. Lille.

——— (1911 *a*): Nouvelles remarques sur la fronde des Zygoptéridées. Bull. Soc. Hist. Nat. Autun, xxv.

——— (1911 *b*): Structure des stipes d'*Asterochlaena laxa*. Mém. Soc. Géol. du Nord, t. vii, mém. No. 1.

——— (1911 *c*): Observations sur les Cladoxylées. Assoc. Franç. pour l'Avancement des Sciences, 40e session, Dijon, p. 509.

——— (1912): L'étude anatomique des fougères, etc. Progressus rei botanicae, iv.

——— (1914): État actuel de nos connaissances sur les genres *Cladoxylon* et *Steloxylon*. Assoc. Franç. pour l'Avancement des Sciences, 43° session, Le Havre, p. 446.

CORDA (1845): Beiträge zur Flora der Vorwelt. Prag.

GOEPPERT, H. R., und STENZEL, G. (1881): Die Medulloseae, eine neue Gruppe der fossilen Cycadeae. Palaeontographica, 28 (3. Folge, 4. Lief., iii), p. 16.

GORDON, W. T. (1911 *a*): On *Diplolabis Roemeri* Solms. Trans. Roy. Soc. Edin., xlvii, Part IV, No. 24.

——— (1911 *b*): On *Metaclepsydropsis duplex* Will. Ibid., xlviii, Part I, No. 8.

KIDSTON, R., and GWYNNE-VAUGHAN, D. T. (1910): On the Fossil Osmundaceae. Part IV, ibid.

OSBORN, E. M., MRS. (1915): Preliminary Observations on an Australian *Zygopteris*. Report British Assoc., pp. 727–8.

SAHNI, B. (1918): On the Branching of the Zygopteridean Leaf and its Relation to the Probable 'Pinna' Nature of *Gyropteris sinuosa* Goeppert. Annals of Botany, xxxii.

SCHENK, A. (1889): Ueber *Medullosa*, Cotta, und *Tubicaulis*, Cotta. Abh. d. kgl. sächs. Ges. d. Wiss., xxvi, Math.-Phys. Kl., xv.

——— (1890): Die fossilen Pflanzenreste. Handbuch der Botanik, iv. Breslau.

SCOTT, D. H. (1909): Studies in Fossil Botany.

——— (1912): On a Palaeozoic Fern, the *Zygopteris Grayi* of Williamson. Annals of Botany, xxvi (i), p. 39.

SOLMS-LAUBACH, H., GRAF ZU (1896): Ueber die seinerzeit von Unger beschriebenen . . . Pflanzenreste . . . von Saalfeld. . . . Abh. d. k. preuss. geol. Landesanstalt, Neue Folge, Heft 23, Berlin.

——— (1910): Ueber die in den Kalksteinen des Kulm von Glaetzisch-Falkenberg in Schlesien erhaltenen strukturbietenden Pflanzenreste, IV. Zeitschrift für Botanik.

STENZEL, G. (1889): Die Gattung *Tubicaulis*, Cotta. Bibliotheca Botanica, Heft 12, Cassel.

UNGER, F. (1854): Zur Flora des Cypridinenschiefers. Sitzungsber. d. k.-k. Akad. Wiss. Wien, Bd. xii, p. 599.

——— (1856): Beitrag zur Paläontologie des Thüringer Waldes (Richter u. Unger). Denkschr. d. k.-k. Akad. zu Wien, Math.-Naturwiss. Kl., xi, p. 165.

EXPLANATION OF PLATE IV.

Illustrating Mr. Sahni's paper on an Australian Specimen of *Clepsydropsis*.

(The figures are all from untouched photographs, with the exception of Fig. 3, in which some of the fainter lines on the print were intensified with a lead pencil.)

Fig. 1. Transverse section of a diarch root-strand; the two poles lie at the right and left ends of the strand. × *c.* 35·5.

Fig. 2. Transverse section of peripheral region of a petiole. *o.c.*, outer cortex; *i.c.*, inner cortex. × *c.* 38.

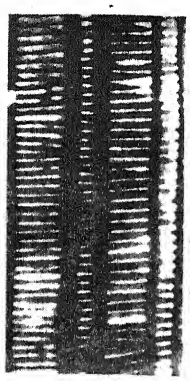
Fig. 3. Longitudinal section of the same. × *c.* 43.

Fig. 4. Longitudinal section of a portion of a petiolar strand, to show the finely scalariform pitting or the tracheides. × *c.* 133.

Fig. 5. Transverse section of a portion of the petiolar strand, also shown in Text-fig. 2, *3*. *Owing to a fracture in the xylem, the peripheral loop appears considerably wider than is normally the case* (*compare with the undisturbed strand shown in Text-fig. 2*). The loop is lined internally as well as externally by specially narrow tracheides. × *c.* 40.

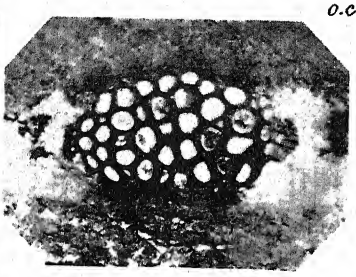
Fig. 6. Transverse section of the petiolar strand, also shown in Text-fig. 2, *9*. *a.*, *a.*, antennae; *p.l.*, peripheral loops; *s.t.*, small tracheides, forming a more or less complete bridge between the two loops. × *c.* 29.

Fig. 7. Longitudinal section of the same strand, passing radially across its median plate, to show the small tracheides, *s.t.*, flanked by the larger tracheides. × *c.* 33.



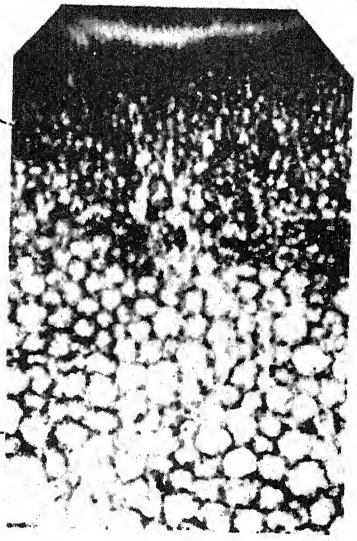
4.

1.



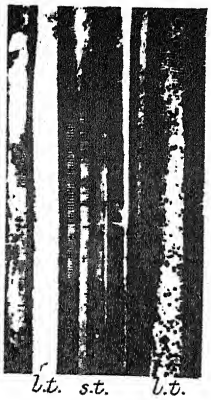
2.

o.c.



i.c.

7.



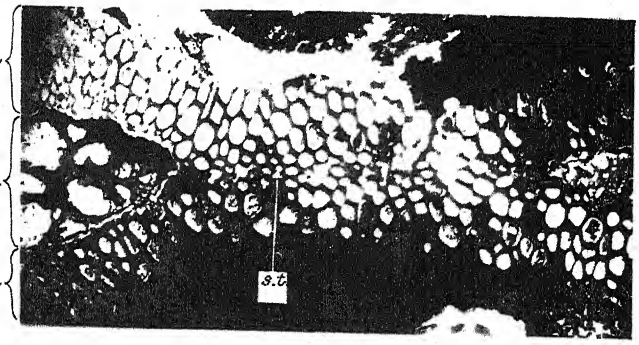
l.t. s.t. l.t.

6.

a

p.l.

a

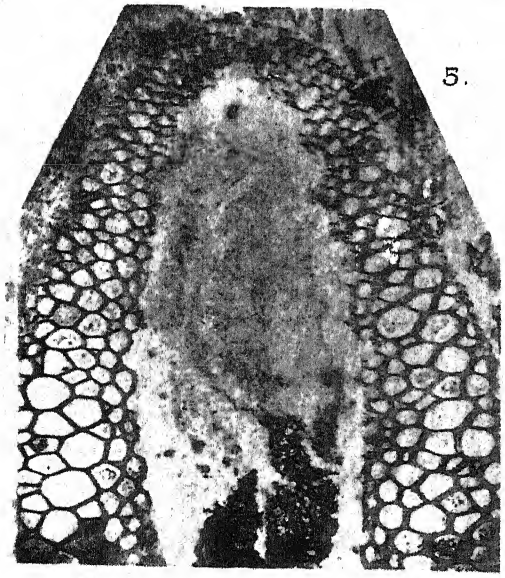


s.t

a

p.l.

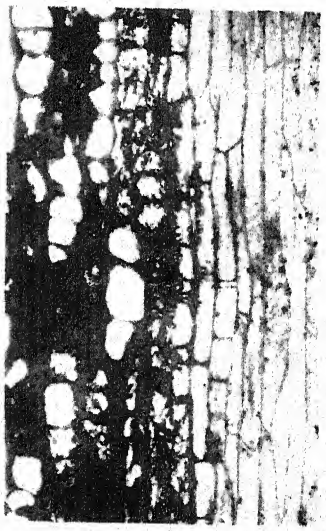
a



5.

B. Sahni phot.

i.c. 3. o.c.



Huth coll.

SAHNI— CLEPSYDROPSIS.

Observations on Euglena deses.

BY

ROSE BRACHER.

With nine Figures in the Text.

CONTENTS.

		PAGE
SECTION I.	INTRODUCTION	93
,, II.	DAILY MOVEMENTS OF *E. DESES* DEPENDENT ON EXTERNAL FACTORS	95
,, III.	BEHAVIOUR OF *E. DESES* WHEN REMOVED FROM THE INFLUENCE OF THE TIDE	99
,, IV.	FURTHER INVESTIGATIONS OF THE INFLUENCE OF LIGHT AND TEMPERATURE ON THE BEHAVIOUR OF *E. DESES*	100
,, V.	DEPTH TO WHICH *E. DESES* BURROWS IN MUD	104
,, VI.	CLIMATIC CONDITIONS	105
,, VII.	THE NEED FOR OXYGEN	106
,, VIII.	SUMMARY	106
,, IX.	CONCLUSION	107
BIBLIOGRAPHY		108

INTRODUCTION.

THE organism with which this paper has to deal occurs along the banks of the river Avon within its tidal portion, which extends for about seven and a half miles inland. The actual point at which the present work was for the most part carried out is about a quarter of a mile lower down the river than the Clifton Suspension Bridge. At low tide the river in this part of its course is reduced to a very narrow stream, leaving exposed on either side wide stretches of mud.

On examining the surface of the mud, one can see that parts are coloured bright green, while in other places the mud appears yellowish brown in hue. Closer examination reveals the fact that the green colour is restricted to small patches varying from a quarter of an inch to one inch in diameter, and often so close together as to form an almost continuous carpet.

[Annals of Botany, Vol. XXXIII. No. CXXIX. January, 1919.]

By examining small quantities of the mud under the microscope, one can see that the green colour is due to the presence of a vast number of small green organisms, identified by the aid of Dangeard (3) and Wager (7) to be *Euglena deses*. The yellow and brown colour is due to the presence of a large number of diatoms, and it is a noteworthy fact that while the Euglenae occupy, for the most part, the higher positions on the ridges of mud, the diatoms are usually to be found in the damper mud of the hollows.

According to Dangeard (4) *Euglena* requires a certain amount of organic matter for its nutrition, and the occurrence of the organism in so great abundance here is probably due to the quantity of sewage brought down to the Avon from the neighbouring towns. An analysis of the mud at this spot shows that the organic content is 6 per cent. (approx.).

By continuing observations on the banks of the Avon, one may see that the green colour mentioned above is not permanently on the mud, but disappears and reappears apparently according to the times of sunrise, sunset, and high tide. One may well compare the behaviour of this organism with that of *Convoluta roscoffensis* described by Gamble and Keeble (5) and Georges Bohn (1). This animal is found on the shores of Brittany, where it colours patches of the sand bright green. It comes to the surface during certain times of the day, but burrows into the sand during darkness and for the periods of high tide. Laurie (6) describes similar diurnal movements in the Peridinian, *Amphidinium operculatum*, which is brown in colour and occurs on the sea-shore at Port Erin. Bohn (2) further shows that certain Gasteropods, such as *Littorina rudis*, exhibit diurnal migrations, taking shelter under pebbles and rocks during darkness and high-tide periods.

Although such comparisons are of great interest, one should remember that in all the organisms mentioned above, with the exception of the Peridinian, there is a much more highly developed structure than in the unicellular *Euglena*, and therefore it is not wise to lay too great a stress on their similarities and differences.

This paper was, therefore, undertaken with a view to investigating the possible influence of such external factors as light, tidal flow, and temperature changes upon the movements of *E. deses* and the part which they play in the life of the organisms.

E. deses is very contractile and constantly changing its shape. By a series of changes, consisting chiefly of contraction into a ball, expansion to full length, and bending round upon itself, the organism is able to creep about over the surface of the mud. Up to the present time no cilia have been observed, and in the experiments performed the organism appears to be unable to swim freely in water. One would expect, therefore, that any response to stimuli which the organism makes would of necessity be much

slower than that made by the free-swimming forms such as *E. viridis*. Fig. 1 shows the changes in shape occurring in one specimen of *E. deses* during several minutes' observation under the microscope.

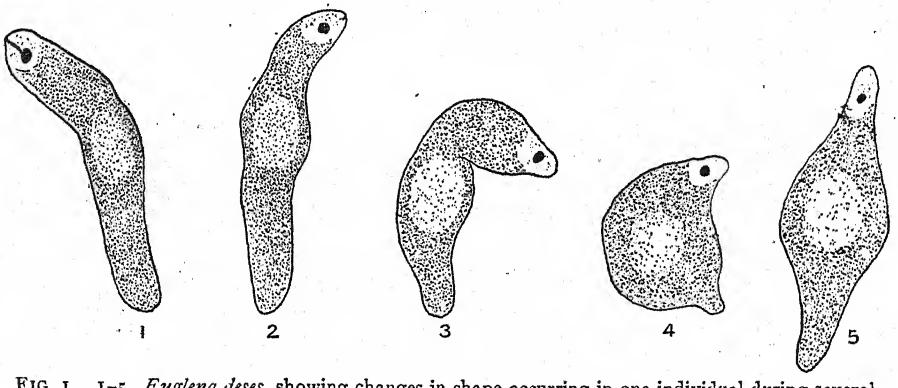


FIG. 1. 1–5. *Euglena deses*, showing changes in shape occurring in one individual during several minutes' observation. (Mag. 400 times.)

DAILY MOVEMENTS OF *E. DESES* DEPENDENT ON EXTERNAL FACTORS.

A series of observations were made on the Avon banks between dawn and sunset with a view to investigating the times of the daily movements of *Euglena*.

At dawn no Euglenae are visible on the surface of the mud, but from readings taken in October, December, April, and May the green colour begins to appear about one to two hours after sunrise.

Month.	*Week.*	*Average Time of Sunrise.*	*Time of Appearance of Euglenae.*
October	8th–15th	6.12	7.30
December	3rd–9th	7.45	9.0
April	23rd–29th	4.37	6.30
May	14th–20th	4.3	6.0

As the light becomes brighter and the temperature increases the mud becomes correspondingly greener, until about midday the surface is very green indeed. The organisms remain thus exposed until the light begins to fade in the evening, when they once more disappear from view and burrow down into the mud. Readings were taken in the same months for the time of disappearance of the organism, which is shown to be less than half an hour before sunset.

Month.	*Week.*	*Average Time of Sunset.*	*Time of Disappearance.*
October	8th–15th	5.15	5.0
December	3rd–9th	3.49	3.30
April	23rd–29th	7.18	7.0
May	14th–20th	7.40	7.30

The diurnal movements would cause one to expect corresponding changes in the appearance of the organism during the day, and accordingly drawings were made at various times to investigate the nature of these.

Early in the morning they appear extended and active as they rise to the surface and are moving with continual amoeboid movements. Later in the day they appear rounded off, and at night the eyespot is completely hidden from view.

If light were the only determining factor the above results would include all the daily movements of *Euglena deses*; but its behaviour, as has already been stated, is considerably modified by the response of the organism to the action of the tide.

If a patch of mud is watched immediately after the tide has receded from it, no Euglenae are visible. From about a half to one hour later the first make their appearance in small isolated groups, about a quarter of an

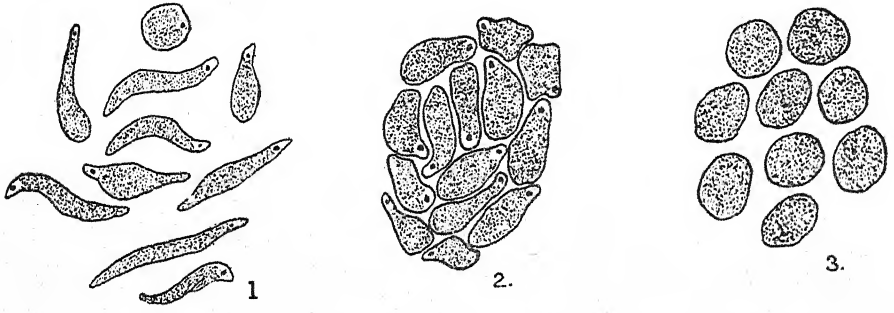


FIG. 2. *E. deses.* Diurnal differences in appearance. (Mag. 100 times.) (1) Early morning (actively moving); (2) midday (lying still on mud surface); (3) night (rounded off).

inch in diameter. With regard to the incoming tide, the Euglenae do not disappear into the mud before they are covered by the tide, but when a few ripples of the rising water have passed over the spot they burrow down, and the green colour quickly fades and does not reappear until after the water has gone back.

It may be seen, therefore, that the intervention of the action of the tide alters to a considerable extent the simple diurnal movements dependent solely upon light, and one would expect to find much variation in the behaviour of the organism according as the two factors, light and tidal action, are reacting simultaneously or at different times.

In order to keep the Euglenae under closer observation and yet under natural conditions, an apparatus was devised for producing a periodic tidal flow over a given area of mud. In main essentials this apparatus consisted of a tank which was filled and emptied automatically by means of siphons which had been so arranged as to produce a tide at the proper time. In Fig. 3 a drawing of the apparatus is shown.

Mud (M) is placed in the glass tank T in the position shown in diagram. Cement dams (C_1 and C_2) serve to keep the mud in position. Water is stored in two vessels (A_1 and A_2) which are connected by the siphon S_1. By means of a tap at the base of the siphon S_2 water is run drop by drop into the bottle B. By keeping this always overflowing, the pressure of water in the bottle remains approximately constant. By means of the tap water from the bottle is then run into the inverted bell-jar J. The indicator I enables the stop-cock to be kept at a definite angle, and

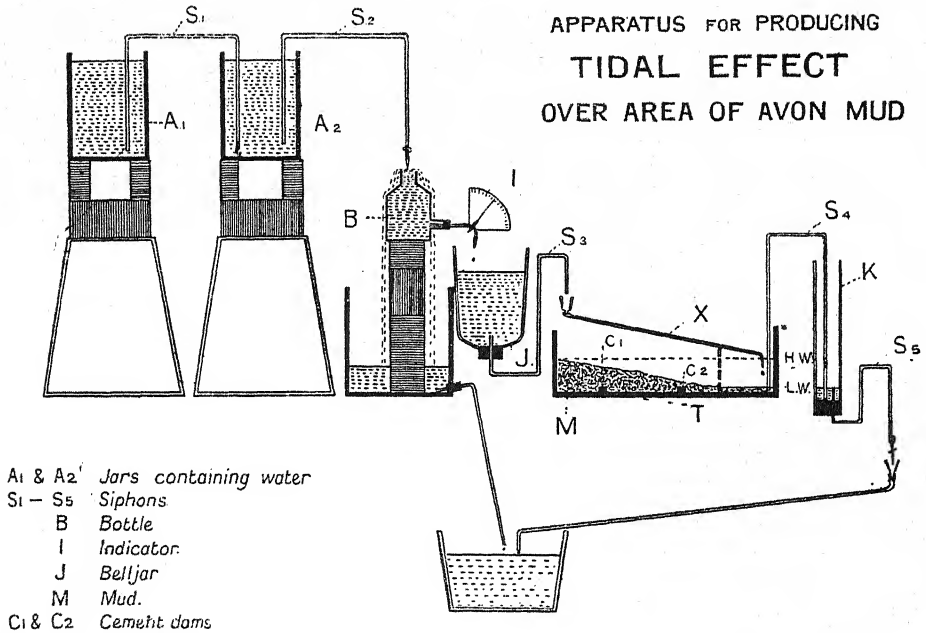


FIG. 3.

by adjusting this the time taken to fill the bell-jar may be regulated. By trial it was possible to fill the bell-jar to the required level in twelve hours. In reaching the level of the siphon S_3 the water all passes over into the tank by means of the tube X, and high tide is effected. The outgoing tide is arranged thus: As the water rises in the tank T it also rises in the glass cylinder K, the two being connected by the siphon S_4. This cylinder is too narrow to allow any appreciable fall of water to take place in the tank. On reaching the level of the siphon S_5 the water in the cylinder is gradually passed off by means of slow dripping from the tap at the end of the siphon, and accordingly the water is gradually emptied from the tank until it reaches the level of the upper end of siphon S_5 within the cylinder, when air enters and the flow of water is stopped. By

regulating the tap the duration of high tide may be altered to suit the experiment.

As the greenness of the mud varied from hour to hour, readings were taken, and for this purpose a colour scale was made consisting of four colours to which the numbers 0, 5, 10, and 15 were given respectively.

Shade 15 = Brightest green colour on mud.
Shade 10 = Green colour shown when Euglenae are beginning to go down.
Shade 5 = Very pale green when nearly all Euglenae have disappeared.
Shade 0 = Pure grey colour of mud.

By practice it was possible to read which shade the mud most closely resembled at the time when the readings were taken. These readings were

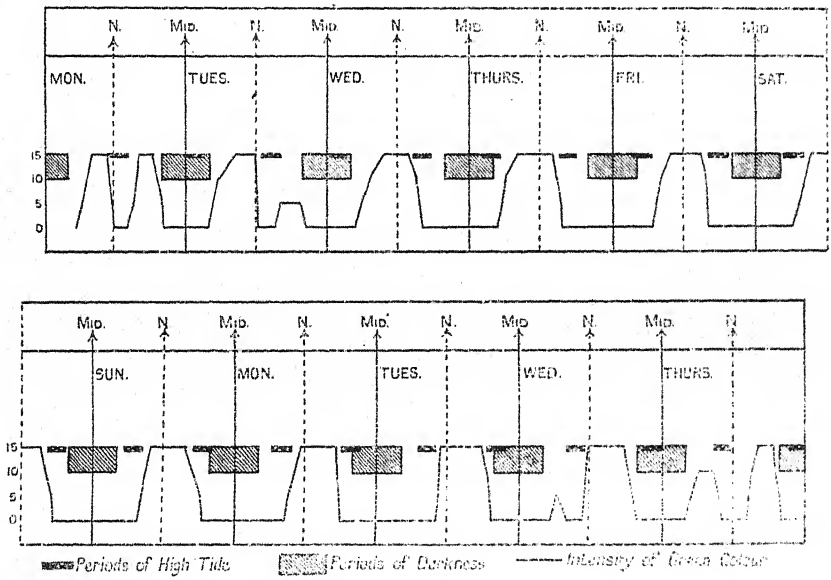


FIG. 4. The graph represents the intensity of greenness on the mud under normal conditions with reference to the periods of high water (depicted black) and of darkness (depicted shaded). The intensity of greenness is measured according to the following scale: 15 (bright green), 10 (green), 5 (pale green), and 0 (pure grey colour of mud). The observation extends over eleven days, the divisions of which are marked at noon (N.) and midnight (Mid.).

plotted on a graph and a curve obtained, while the periods of high tide, and also those of day and night, are shown in order to compare the appearance of the mud with these (Fig. 4).

Starting on *Monday* it may be seen that the organisms make their appearance about two hours after sunrise and attain their maximum number on the surface between the hours of 8 a.m. and 11.30 a.m. About half an hour before the tide comes in they begin to go down and completely disappear

as the water rises over them. About half an hour after the tide has receded they reappear, regain their maximum number between the hours of three and five, and disappear again completely just before sunset. On *Tuesday* the process was repeated, but owing to a decrease in time (about five hours) between the ebb of the tide and the sunset, the Euglenae do not reappear to their fullest extent and the mud does not become very green. On *Wednesday* this period of time is reduced to four hours, and the Euglenae make no reappearance after high tide during that day. On *Thursday* and *Friday* the same phenomenon occurs as the time is still further reduced. On *Saturday* and *Sunday* the appearance of the organisms is retarded, since the period of high tide extends over the usual time when they rise to the surface. On Monday and Tuesday of the second week the periods of time between sunrise and high-water are two and three hours respectively, but the organisms do not rise to the surface until after the tide has receded. On Wednesday the period is increased to four hours, and on this day the Euglenae appear on the surface in small numbers for a short time before high tide. On Thursday the period between sunrise and high water is still further prolonged, and the Euglenae appear for a longer time and in greater numbers, but it is not until the tide once more approaches the region of midday that the green colour on the mud attains its maximum degree of intensity twice in one day.

Thus it may be seen:

1. That, other conditions being equal, the Euglenae rise to the surface about two hours after sunrise and go down shortly before sunset, burrowing into the mud during the day for the period of high tide.

2. That if the period between the ebb of the tide and sunset is not longer than about four hours, the organisms do not reappear during this interval.

3. That if the period between sunrise and high water is not longer than three hours, the organism does not appear on the surface until the tide has receded.

Behaviour of *E. deses* when removed from the Influence of the Tide.

It may easily be seen that those Euglenae which live close to the outer edge of the river bank are only covered by the tide for comparatively short periods, while those lower down may remain covered with water for several hours. Moreover, owing to the fortnightly variations in the tide, it happens that during neap tides this outer zone may be exposed for several days without being covered by the tide. Material was taken from this region and kept in dishes, and it was noticed that the organisms responded only to the stimulus of light. In other material, however, which was taken from

a zone always under tidal influence, it was noticed that, although removed from the Avon and placed in a dish in the laboratory, the organisms still continued to burrow into the mud at the time of high tide.

In order to ascertain exactly how long this phenomenon persisted, accurate readings were taken at every hour by means of the colour scale used in the previous experiment, and curves drawn in order to show the behaviour of the organisms when removed from the influence of the tide (Fig. 5).

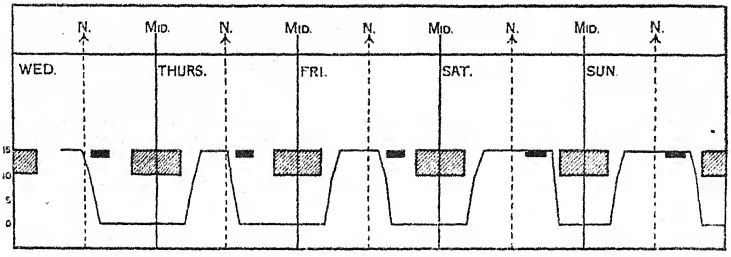


Fig. 5. A graph drawn to illustrate the behaviour of *E. deses* when removed from the influence of the tide. The lettering and shading are as in Fig. 4.

The material was obtained at 9 a.m. on Wednesday morning, when the mud was well covered with Euglenae. It was placed in the laboratory and left without any tidal influence.

Result.

On Wednesday afternoon the organisms went down during the period of high tide.

On Thursday afternoon the organisms again went down at about the same time as the period of high tide on Wednesday.

On Friday the organisms again went down for the same period, though they commenced to do so about an hour later, the disappearance being more rapid.

On Saturday and Sunday, however, the Euglenae did not disappear at all during the day until just before sunset.

Therefore, although removed from tidal influence, *E. deses* continues to show tidal periodicity for about three days. Gamble and Keeble (5) describe a similar periodicity in *Convoluta*, which they maintain only lasts for one day. Bohn (1), however, states that he has watched the phenomenon during fourteen consecutive tides.

Further Investigations of the Influence of Light and Temperature on the Behaviour of *E. deses*.

The influence of light. It has been shown that *E. deses* is sensitive to light, since it comes to the surface of the mud as soon as the daylight has reached a sufficient degree of intensity. Small variations in the light during

the day appear to make no difference to the number of Euglenae on the surface, as in bright sunlight or good daylight without sun the greenness of the mud remains the same. If, however, the day is extremely dull and the intensity of the light does not reach a certain point required by the Euglenae, only a very few organisms appear, and from December 13 to December 15, when a very heavy fog enveloped the city, no individuals could be seen on any of the mud under observation. The Euglenae disappear into the mud at nightfall, but the same phenomenon can be brought about by placing them at any time in the dark. It was found that the time taken for complete disappearance varied with the temperature, as will be shown later, but averaged about thirty minutes. On replacing in the light the Euglenae took about the same time to rise to the surface again.

If kept in the dark for one or two days the Euglenae do not appear on the surface until replaced in daylight. If kept for longer periods a few may be visible on the surface of the mud, and eventually most of them return to the surface, probably to obtain more oxygen. The chief difference in the appearance of these Euglenae kept in the dark is the disappearance of the paramylon grains which are used as reserve food, and when all has been used up the organism dies. On being replaced in light, however, before all the paramylon has disappeared, the *Euglena* is able to manufacture fresh grains in a very short period of time.

Another experiment was performed in which the Euglenae were placed in dishes and illuminated only from one side in order to see if they would move towards the light.

Four dishes were used which contained respectively:

(1) A quantity of stiff mud containing Euglenae.

(2) A quantity of wet mud containing Euglenae.

(3) A quantity of mud with Euglenae, over which a layer of water half an inch deep was placed.

(4) A number of Euglenae in water only.

In (1) and (2) the Euglenae gradually moved towards the source of light, and about the end of five days were all massed at the lighter end of the dish, many heaped upon the glass.

In (3) the Euglenae moved somewhat towards the source of light, but not so pronouncedly as in (1) and (2).

In (4) the organisms kept their original position, and at the end of the day appeared completely dead, owing to a lack of oxygen and food material, as they are not able to swim in water.

In the diagram given below (Fig. 6) the positions of the Euglenae at the beginning and end of the experiment are shown.

From these experiments it may be concluded that *Euglena deses* is sensitive to light and moves towards the source of light, and is not repulsed by bright sunshine.

Its movements in response to the stimulus are very slow as compared with results obtained by Wager (8) on *Euglena viridis*. He mentions that if a cloud passes over the sun these organisms immediately leave the position which they occupy on the surface of a pond and swim down into the water, to reappear as soon as the cloud has passed. Also a number

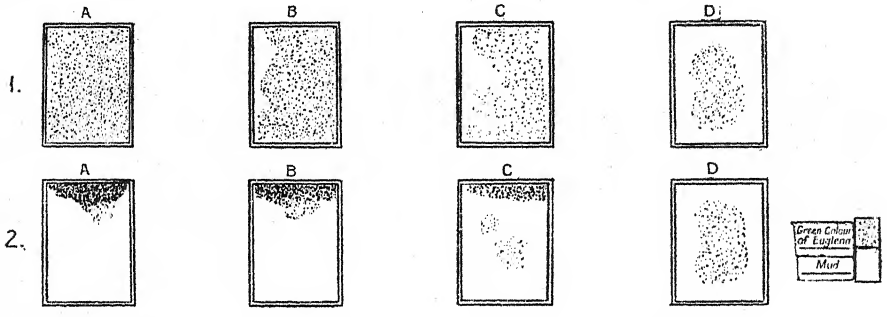


FIG. 6. (1) Position of Euglenae at commencement of experiment: A, stiff mud; B, wet mud; C, mud covered by water; D, water only. (2) Position of Euglenae after five days' illumination from one side.

of Euglenae in a saucer illuminated from one side will pass towards the source of light in a few minutes.

Influence of temperature. Cultures of *Euglena* were kept at temperatures ranging from 3° C. to 20° C., and in these the organisms appeared to flourish equally well. By changing the temperature, however, one is able to observe a marked difference in the rate at which the organism responds to stimuli.

At low temperatures, i. e. below 4° C., it was found that the Euglenae became rounded off, very sluggish, and slow in response to stimuli, but as the mud was gradually warmed up, they became more active and expanded to their fullest length.

During the winter of 1916–17 the periods of frost were very severe. From December 6 to December 17 the average minimum temperature was −3° C. The mud in the vessels was frozen each night, but during the warmest part of the day, when the mud thawed and rose to a temperature of 3° C., Euglenae were visible in small numbers for a few hours.

From January 20 onwards the mud was completely frozen for a period of twenty-nine days. The temperature frequently fell as low as −10° C., and the mud did not thaw at all during the day-time. When the thaw did set in, however, the mud was closely watched for any traces of Euglenae either in the vegetative or encysted form. Five days later, a faint green colour on the mud showed, on examination, that some of the Euglenae had withstood the severe frost.

Experiments were then performed in order to see at what temperature the Euglenae become active again after freezing. Four dishes containing mud and Euglenae were placed out of doors at nightfall so that the next morning the mud in all the dishes was frozen. They were placed in a good light and kept at different temperatures as follows:

(1) Kept all day at 0° C.
(2) „ 1° C.
(3) „ 2° C.
(4) Warmed slowly from 0° C. to 4° C.

These experiments were repeated several times and the following results obtained:

In (1), (2), and (3) no Euglenae appeared during the day.

In (4) the first traces of Euglenae appeared on the surface of the mud when the temperature was approximately 2·5° C.

If the mud is frozen while the organisms are visible and then placed in

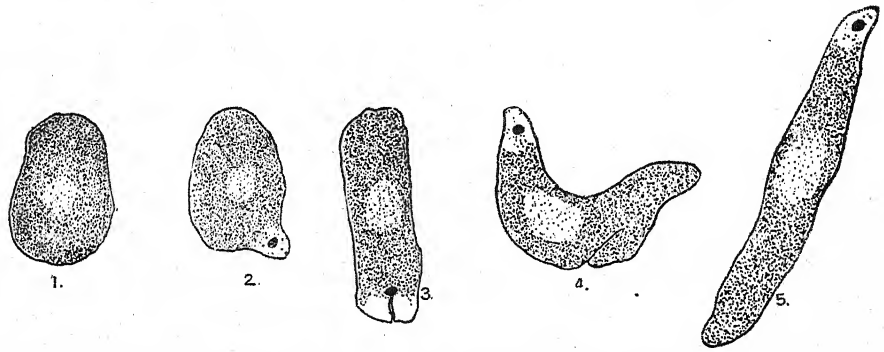


FIG. 7. *E. deses.* 1–5, changes in shape of one individual when warmed from 1·5° C. to 16° C.

the dark, the Euglenae will not burrow into the mud until the temperature is as high as 2·5° C. At low temperature the Euglenae appear rounded off, and Fig. 7 shows the changes in appearance when the organism is warmed.

In order to see at what temperature the organism is most active the cultures were kept at different temperatures and covered with a black cloth, and the time taken for complete disappearance was recorded. From a large number of experiments performed, the following results were obtained:

Temperature.	*Time to disappear.*
3° C.	60 minutes (approx.)
6° C.	40 „ „
9° C.	30 „ „
12° C.	25 „ „
15° C.	20 „ „
20° C.	30 „ „
25° C.	1½ hours

A graph showing these results is given in Fig. 8.

E. deses is therefore capable of withstanding extremes of temperature for short periods, but is less able to withstand long periods of adverse conditions. The organisms become very sluggish if subjected to temperatures above 25° C. or below 5° C., and below 2·5° C. no response to stimuli has been observed. The rate at which the organism responds to stimuli is

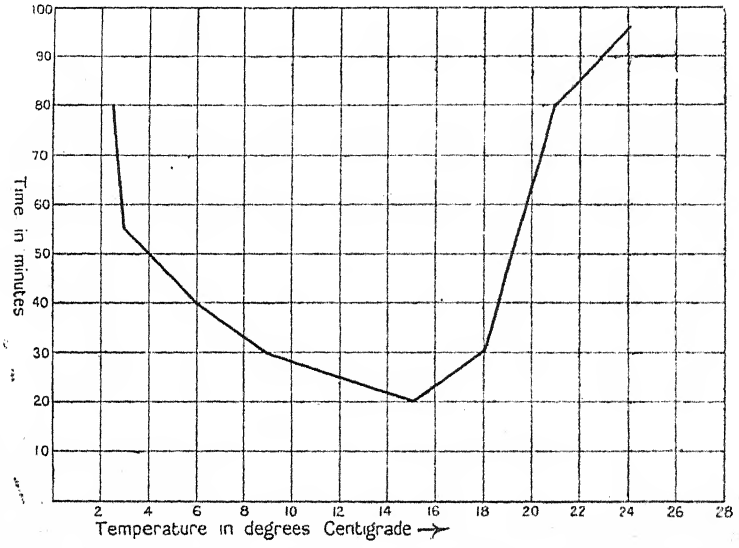


FIG. 8. Graph showing the time taken for *Euglena deses* to respond to the stimulus of light at different temperatures.

dependent on the temperature. It is increased as the temperature is raised, but above 15° C. its activities grow less.

DEPTH TO WHICH *E. DESES* BURROWS IN MUD.

Experiments were performed with a view to finding the depth the Euglenae burrow in the mud in response to stimuli. By means of a Gillette razor-blade the surface mud was scraped off in several dishes to a depth of $\frac{1}{8}$ in., $\frac{1}{4}$ in., and $\frac{1}{2}$ in. respectively. The time chosen was after the cultures had been placed in the dark, i. e. when the Euglenae had burrowed into the mud.

As a result it was found that in the $\frac{1}{8}$ inch experiment a large number of Euglenae reappeared when placed in the light, in the $\frac{1}{4}$ inch a few, and in the $\frac{1}{2}$ inch none at all.

Therefore one would conclude that the majority of Euglenae do not burrow deeper than $\frac{1}{4}$ inch. The experiment was repeated by inserting a strip of Bolton silk, mounted on a frame at an angle of 45°, in the mud. The organisms burrowing down became entangled in the meshes, and by

measuring the distance down the slope over which the organisms are to be found one is able to calculate the greatest distance to which they burrow.

As a result it was found that the greatest depth was about $\frac{1}{2}$ inch, while the majority were found at about $\frac{1}{8}$ inch. This agrees with the results obtained in the previous experiment, and also with that obtained by Laurie for *Amphidinium*, which he says burrows to a depth of about $\frac{1}{8}$ inch (6).

Climatic Conditions.

(1) *Rain.* Several visits paid to the Avon during wet weather show that *E. deses* does not appear on the actual surface of the mud during rain. If, however, one examines the sides of the small drainage channels which

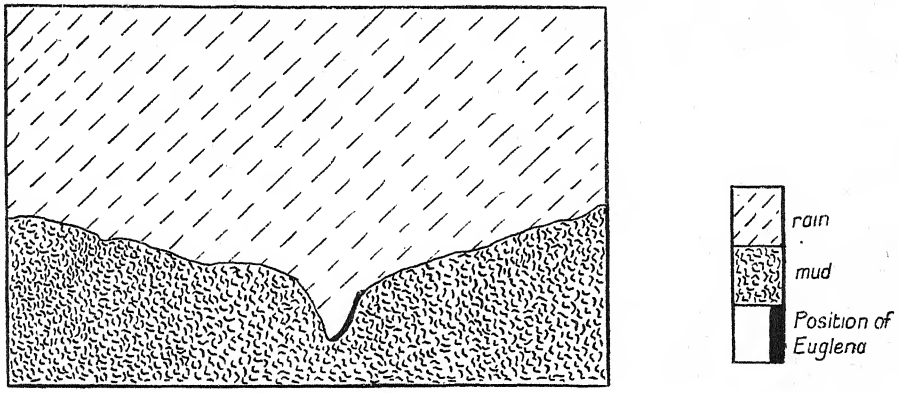


Fig. 9. Showing position taken up by Euglenae on the mud during rain.

run through the mud at right angles to the river, one can see a large number of Euglenae clustered here to obtain shelter from the rain, and it is a noteworthy fact that they occur in far greater numbers on the side from which the rain is driving and so are almost in complete shelter. A section drawing is shown in Fig. 9.

(2) *Drought.* The mud at the outer edges of the river bank often becomes very dry when the tide does not cover it for some days, and in order to see the effect of drying on the organism, mud was kept in dishes and left until quite dry and hard.

On examination it was found that all the Euglenae were dead and disorganized.

Other mud was kept until it became very stiff but was still soft enough to receive an impression. With this the Euglenae ceased to respond to the stimulus of light and remained on the surface of the mud. They appeared rounded off and dark green in colour. On subsequent wetting the organisms

regained their activity and were capable of responding to the light stimulus. In none of the experiments was found any sign of encysted forms.

E. deses is therefore able to withstand drying up to a certain point, but as soon as the mud becomes hard and crumbles, the organism is apparently killed.

The Need for Oxygen.

It has been shown that *E. deses* will not live in stagnant water. This is probably owing to the lack of suitable food and partly to the lack of oxygen.

In order to investigate the second factor separately, a dish containing mud and Euglenae was placed under a bell-jar with pyrogallic acid and potash. The bell-jar was sealed to the glass plate on which it stood by means of wax.

It was noticed that after three days the organisms ceased to come up and down in response to the stimulus of light, and on examination were seen to be contracted into very irregular shapes. After a few minutes' exposure to the air they regained their normal appearance.

A second experiment was carried out in which the organisms were left without oxygen for a week, when the organisms were contracted but dead, and did not revive on exposure to air.

Thus when oxygen becomes scarce *Euglena* is able to resist this adverse condition for a time by contraction, but if completely deprived of oxygen for some days life is impossible.

Summary.

The behaviour of *E. deses* living on the banks of the Avon is dependent on certain external factors, namely, light, tidal flow, and temperature changes.

The influence of light. This influence is a direct one and therefore of great importance. The Euglenae are visible on the mud during day-time, but burrow under the surface during the night, which phenomenon can be brought about by placing the organisms at any time in darkness.

The influence of tidal flow. The tidal influence shows itself inasmuch as the organism burrows into the mud during the period that it is covered by the tide. If the time of high water is not more than three hours after sunrise the appearance of the organism is delayed, and correspondingly, if the time of high water is not more than four hours before sunset, the re-appearance of the *Euglena* does not take place that day. Further, *E. deses* possesses a tidal periodicity which causes it to respond to the stimulus for about three days after being removed from the actual tidal influence.

The influence of temperature changes is not so important as those

mentioned above, as *E. deses* is active at any temperature between 2·5° C. and 25° C. Outside these limits its movements are arrested. By raising the temperature the movements of the organism are accelerated within certain limits, the optimum temperature being about 15° C.

Conclusion.

One would conclude from the foregoing experiments that the life of *Euglena deses* is dependent on a series of movements which the organism performs each day. With regard to the physiological reasons for such movements very little can be said, but it is worthy of note that the two series of movements, namely, those in response to light and those in response to the tide, are different in nature. The influence of light is a direct one, for Euglenae placed in the dark at any time disappear, to reappear when replaced in the light. There is no evidence of any periodic effect, for in cultures placed in the dark overnight and left there during the next day the Euglenae do not reappear at the accustomed time in the morning. As soon, however, as the culture is replaced in the light the organisms come to the surface. From this one would think that the periodic tidal movement is not dependent on light conditions, but is rather due to the shock of the waves, as is thought by Bohn (2) to be the case in *Convoluta roscoffensis*.

Finally, it is very interesting to observe how the requirements of such an organism as *E. deses* are so well suited to the conditions under which it lives. Being unable to swim freely in water, it cannot live at the bottom of ponds or stagnant pools, or it would be unable to obtain sufficient oxygen to carry on its life processes, and on the other hand it is unable to withstand drought and could not live in mud which is liable to become completely dried in the summer. The periodic tidal flow is therefore an ideal condition, since the organism is kept moist and has also long periods of exposure when it is able to carry on respiration to a greater extent than when covered by water. The tide tends to keep the mud at fairly equable temperatures, and this is of distinct advantage, since the organism is unable to withstand extremes of temperature.

Being thus so well adapted to its mode of life, the organism is able to exist in the vegetative state all the year round, and this may account for the fact that no encysted forms, so common in *E. viridis* during dry seasons, have as yet been observed here.

In the struggle for existence the organism has evolved a structure which enables it to live in an habitat where few other organisms occur, and so the seasonal dying out of the species, which occurs in ponds when one form of organism supplants another, plays no part here, and the organism is present in immense numbers all the year round.

I take this opportunity of offering my best thanks to Dr. O. V. Darbishire, under whose direction the work was carried out, and also to Professor Gamble, F.R.S., and Miss E. M. Lee, M.Sc., for valuable help and advice.

DEPARTMENT OF BOTANY,
UNIVERSITY OF BRISTOL.

BIBLIOGRAPHY.

1. BOHN: Sur les mouvements oscillatoires de *Convoluta roscoffensis*. Comptes rendus des Séances de l'Académie des Sciences, vol. cxxxvii, 1903, pp. 576–8.
2. ——: Périodicité vitale des animaux soumis aux oscillations du niveau des hautes mers. Comptes rendus des Séances de l'Académie des Sciences, vol. cxxxix, 1904, pp. 610–11.
3. DANGEARD: Recherches sur les Eugléniens, pp. 92–9.
4. ————: Ibid, p. 197.
5. GAMBLE and KEEBLE: The Bionomics of *Convoluta roscoffensis*. Quarterly Journal of Microscopical Science, vol. xlvii, 1903.
6. LAURIE: On the Bionomics of *Amphidinium operculatum*. Report of British Association, 1913.
7. WAGER: On the Effect of Gravity upon the Movements of *Euglena viridis* and other Micro-organisms. Phil. Trans. Royal Society, London, vol. cci, pp. 333–90.
8. ———: Movements of Aquatic Micro-organisms in Response to External Forces. The Naturalist, June and July, 1914.

A Study of Apogamy in Nephrodium hirtipes, Hk.

BY

W. N. STEIL,

University of Wisconsin, Madison, Wisconsin, U.S.A.

With Plates V–VII.

INTRODUCTION.

THE first reported observation of a sporophyte tissue element in the cells of a fern gametophyte was made by Leszcyc-Suminski (1848), who found tracheides near the apical notch of the prothallium of *Pteris sulcata*, L.

Wigand (1849) described a cylindrical or conical process produced as an outgrowth from the apical notch of the fern prothallium, instances of this sort being found in several cultures. According to his description, the embryo begins its development on the ventral side of the prothallium and at the posterior portion of the process. Since there can be no certainty regarding the purity of his cultures, the peculiar developments described cannot be referred to any particular species. There can be no doubt, however, from his descriptions (l.c., pp. 106, 107) and figures (Figs. 25–9) that he studied embryos of apogamous origin.

Mercklin (1850) found cells in the prothallium of *Pteris sulcata* like those observed by Leszczyc-Suminski. Mercklin also figured (l.c., Pl. VII, Fig. 6) a prothallium and a young embryo of *Nothochlaena Eckloniana*. Mercklin's figure, although somewhat diagrammatic, suggests that the embryo had been apogamously produced. Over a half-century later Woronin (1907) found apogamy in the same species.

Farlow (1874) discovered apogamy in *Pteris cretica albo-lineata*, and was first to recognize clearly the nature of an apogamous embryo as distinguished from one resulting from fertilization. He described the origin of the embryo as an asexual outgrowth from the prothallium. When the embryo is about to begin its development, the portion of the prothallium posterior to the apical notch becomes pale, and tracheides appear in the nearly colourless region. A process, similar to that described by Wigand, develops from the apical notch. The embryo is now produced on the ventral surface of the prothallium posterior to the apical region. The leaf

[Annals of Botany, Vol. XXXIII. No. CXXIX. January, 1919.]

appears first, then the root, and later the stem. A foot is never formed. Between the cells of the gametophyte and those of the apogamous embryo there is no sharp line of distinction. The vascular system of the embryo is intimately connected with vessels in the prothallium. These characters described by Farlow for *Pteris cretica* have been found by other investigators in a number of apogamous ferns. In the same year Abbot (1874) reported apogamy in an unidentified fern.

De Bary (1878) extended Farlow's observations on *Pteris cretica* and also tested a large number of other species to determine the extent of apogamy in ferns. He found it to occur in *Aspidium falcatum* and *A. Filix-mas* var. *cristata*. The development of the embryo in both these species is similar to that described by Farlow for *Pteris cretica*.

Sadebeck (1879) reported apogamy in *Todea africana*. Leitgeb (1885) reported it as occurring occasionally in *Osmunda regalis* and *Ceratopteris thalictroides*. His observations on the two latter species have, however, never been confirmed. Stange (1887) discovered apogamy in *Doodya caudata*, *Todea pellucida*, and *T. rivularis*. Berggren (1888) described it in *Nothochlaena distans*.

The development of the apogamously produced embryos of *Doodya caudata* was investigated by Heim (1896). Since archegonia and antheridia appear to develop to maturity in this species, Heim believed that fertilization may also occur. Archegonial and antheridial 'projections' are developed on the prothallia, and these projections produce the apogamous embryos. As many as thirty projections may be formed on a single prothallium, but only a few embryos produced will survive.

Bower (1888) discovered apogamy in *Trichomanes alatum*. Although spores are ordinarily formed in this species, apospory commonly occurs. The prothallia thus developed give rise to the apogamous embryos.

Lang (1898) induced apogamy in the following species of ferns, the prothallia of which were grown under special cultural conditions for a period of two and a half years: *Scolopendrium vulgare*, Sw., vars. *ramulossisimum*, Woll., and *marginale*, *Nephrodium dilatatum*, Desv., var. *cristatum gracile*, *Nephrodium Oreopteris*, Desv., var. *coronans*, Barnes, *Aspidium aculeatum*, Sw., var. *multifidum*, Woll., *A. angulare*, Willd., vars. *foliosum multifidum* and *acutifolium multifidum*, *A. frondosum*, Lowe, *A. thyrium*, Mett., var. *nipponicum* and var. *cristatum*, *A. Filix-foemina*, Bernh., vars. *bercristatum*, Cousens, *cruciato-cristatum* and *coronatum*, Lowe, and *Polypodium vulgare*, L., var. *grandiceps*, Fox. By watering the cultures from below and keeping them in strong light, Lang believed that apogamy was induced. He was inclined to think that the high temperature under which the prothallia were grown may have been a factor in inducing the appearance of the sporophytic structures, although he believed that the prevention of fertilization was the most important condition.

In all the ferns which Lang placed under these cultural conditions, excepting *Aspidium angulare*, Willd., var. *acutifolium multifidum*, apogamy was induced. He believed that if the prothallia of this variety had been maintained under the cultural conditions for a longer period of time, apogamy might have resulted. In all species excepting *Polypodium vulgare*, archegonial projections were produced. From processes that appeared on the prothallia of *Scolopendium vulgare*, Sw., var. *ramulossisimum*, Woll., and *Nephrodium dilatatum*, Desv., sporangia were formed. The apogamous formation of sporangia in these cases is analogous to the development of antheridia directly on the fern leaf. A case of apospory of this nature was later described by Woronin (1907) in *Trichomanes Kraussii*. The cultural conditions which Lang maintained were evidently not uniform, since in every culture some embryos were produced as a result of fertilization. No control cultures were used by Lang, and, although he exercised great care in his cultural work, no positive conclusion can be drawn from his results regarding the actual conditions which induced apogamy.

In a preliminary note, Farmer, Moore, and Digby (1903) reported nuclear migrations and fusions in the vegetative cells of the prothallium of *Lastraea pseudo-mas*. A diploid nucleus was thus established in some of the cells by a fusion in pairs of the nuclei of adjacent cells. The apogamous embryo, according to their description, begins its development from cells whose nuclei have the double number of chromosomes, the latter being acquired as a result of the 'irregular' fertilization or 'substitution' fusions. Such fusions, as described by these investigators, are the first to be reported in ferns.

Miss Digby (1905) found no reduction in chromosome number in the life-history of the apogamous *Nephrodium pseudo-mas*, Rich., var. *cristata*, fifty chromosomes being counted in the cells of both generations. Nuclear migrations were found in 73 per cent. of the prothallia of the apogamous *Nephrodium pseudo-mas*, Rich., var. *polydactyla*, Wills.

Farmer and Digby (1907) later published a more extensive account of their cytological investigations of apospory and apogamy in ferns. Of the seven forms studied by them, the following were parthenogenetic and aposporous: *Athyrium Filix-foemina* var. *clarissima*, Bolton, *A. Filix-foemina* var. *unco-glomeratum*, Stansfield, and *Scolopendrium vulgare*, var. *crispum Drummondae*. The prothallia of *Athyrium Filix-foemina* var. *clarissima*, Jones, and *Lastraea pseudo-mas* var. *cristata apospora* were produced aposporously. In the former archegonia were produced, but the embryo, as also in the latter species, always originated as a vegetative outgrowth of the gametophyte. In *Lastraea* (*Nephrodium*) *pseudo-mas* var. *polydactyla*, Wills, and *L. pseudo-mas* var. *polydactyla*, Dadds, the prothallia were produced from spores. Archegonia were never formed, and the embryo

in these, as in the two previously mentioned forms, originated as a vegetative outgrowth.

So far as the nuclear history of apogamous ferns is concerned, that of the *polydactyla* varieties is of great interest. Nuclear migrations and fusions were observed by Farmer and Digby to occur between adjoining cells posterior to the apical region of the prothallium. From such a fusion or 'irregular fertilization' the apogamous embryo originated. In all the cases of induced apogamy, Farmer and Digby assume that similar migrations and fusions occur previous to the formation of the embryo. This conclusion is based not alone on their investigations, but on the observations of Lang (1898), who found binucleate cells in the prothallia of some of the ferns in which he believed that he had induced apogamy (his Fig. 2, Pl. VIII). Heim (1896) also figured such cells in the prothallia of *Doodya caudata* (p. 338, Fig. 7). Stephens and Sykes (1910) found binucleate cells in the prothallia of *Pteris droogmantiana*, which, however, resulted from a nuclear division not followed by cell division. Although Stephens and Sykes assumed the occurrence of apogamy in *Pteris droogmantiana* in their preliminary report, so far as I am aware no further contribution has yet appeared dealing with apogamy in this fern.

Farmer and Digby (1907) made a detailed study of the chromosome number in the apogamous and parthenogenetic species. In *Athyrium Filix-foemina* var. *clarissima*, Bolton, eighty-four chromosomes were counted in both generations. Ninety chromosomes were found to be present throughout the life-history of *A. Filix-foemina* var. *clarissima*, Jones, and one hundred were counted in both generations of *A. Filix-foemina* var. *unco-glomeratum*, Stansfield. In each of these cases the number of chromosomes present is, they conclude, presumably the sporophytic number. The nuclei of the prothallial cells of *Scolopendrium vulgare* var. *crispum Drummondae* contain eighty chromosomes, but one hundred were found in the cells of the embryo. Between sixty and seventy-eight chromosomes—probably the gametophytic number—were observed in the nuclei of the aposporous *Lastraea pseudo-mas* var. *cristata apospora*. From sixty-four to sixty-six chromosomes were found in nuclei of the gametophyte of *Lastrea pseudo-mas* vars. *polydactyla*, Wills and Dadds, and from one hundred and twenty-eight to one hundred and thirty-two in the sporophytic nuclei. The double number was, according to their description, established by the fusions of the nuclei in the prothallial cells.

Goebel (1905) found apogamy to occur in *Trichomanes Kraussii* and *Pellaea nivea*.

Woronin (1907, 1908) discovered apogamy in *Pellaea tenera*, *P. flavens*, *Nothochlaena Eckloniana*, and *N. sinuata*. Woronin investigated these cases and also the apogamy of the species reported by Goebel. Archegonia were never produced on the prothallia of any of these species, although normal

antheridia were produced in all cases except *Trichomanes Kraussii*, in which they aborted before reaching maturity. Woronin holds that the *Pellaea* and *Nothochlaena* species have become apogamous on account of the xerophytic conditions under which they live. It is assumed that such an environment, being unfavourable for fertilization, may have led to the development of apogamy.

Yamanouchi (1908 c) reported the occurrence of apogamy in *Nephrodium molle*, Desv., the prothallia of which had been watered from below and kept in direct sunlight. He had shown that fertilization and sporogenesis normally occur in this fern, and hence was certain that the apogamy which he observed had been induced by the cultural conditions. The apogamous sporophyte is described as having the gametophytic number of chromosomes, namely from sixty-four to sixty-six. It is to be regretted that none of the sporophytes thus produced were grown to maturity. A study of sporogenesis, if spores were formed at all, would be of especial interest. According to Yamanouchi, the apogamous sporophyte began its development in a comparatively short period after the prothallium assumed the heart-shaped form and when the archegonia are ordinarily produced. Hence a long period of time does not appear to be necessary to induce apogamy, as seemed to be the case in Lang's experiments.

Miss Black (1909) has duplicated so far as possible with the same species the cultural conditions used by Yamanouchi for inducing apogamy in *Nephrodium molle*. She also grew the prothallia of *Dryopteris stipularis* under the same conditions. Although the prothallia of both species were watered from below and kept in strong light, they developed to a large size and produced sex-organs. At no time were sporophytes produced in any of the cultures so treated.

Mottier (1915) also attempted to show the effect of dry cultural conditions and strong light on the development of apogamous embryos. He grew the prothallia of both the species which Miss Black had used, as well as those of *Onoclea Struthiopteris*. Mottier also was unable to induce the appearance of a single apogamous sporophyte. On the basis of this work and of a study of the normally produced embryo of *Nephrodium molle*, Mottier questions whether Yamanouchi was actually dealing with an apogamously produced embryo.

Heilbronn (1910) found among the prothallia of a culture of *Asplenium Ruta-muraria* prothallia which he identified as those of *Cystopteris fragilis*. The latter were isolated, and their further development was studied. It was observed that in the stronger illumination of summer, embryos were produced apogamously as outgrowths both of the vegetative cells of the prothallium and of the sex-organs. Later in the season, when the illumination was weaker, the projections like those which during the summer formed

embryos now produced antheridia and archegonia, and sporophytes developed only as a result of fertilization. The following summer the new projections again formed apogamous embryos. Heilbronn attributed the occurrence of apogamy to intense illumination, although he failed to obtain similar results in some other species placed under the same conditions. Some differences were observed between the two types of embryos of *Cystopteris fragilis*, but it was not determined whether the spores produced by the apogamously formed sporophytes would produce prothallia of the normal *Cystopteris* form, or those of the apogamous form which Heilbronn distinguished as *Cystopteris* forma *polyapogama*. Heilbronn also found apogamy in *Aspidium aculeatum* var. *cruciato-polydactylum*, Jones, and *A. angulare* forma *grandidens*, Moore.

In several instances Miss Pace (1913) found on prothallia, probably of a species of *Osmunda*, sporangia similar to those described by Lang (1898). The prothallia had been grown for a long period, but were not subjected either to dryness or to strong illumination. She considered the prevention of fertilization by watering from below to be the cause of the formation of the apogamous embryos.

Nagai (1914) obtained apogamous embryos of *Asplenium nidus avis* when the cultures were lacking in moisture, but he believed that high temperature and strong light may also be factors in inducing apogamy.

Miss Allen (1911) studied the cytology of *Aspidium falcatum*, in which de Bary had discovered apogamy in 1878. According to her description there is a fusion in pairs of the sixteen cells which ordinarily in the Polypodiaceae function as the spore mother-cells. The eight cells produced by this fusion function as the spore mother-cells. By the nuclear fusion the double chromosome number is established, but the reduction division follows the fusion, so that the thirty-two spores which are finally formed contain the haploid number of chromosomes.

In a preliminary note (Steil, 1915 b) I have reported a course in *Nephrodium hirtipes* similar to that described by Miss Allen. Further studies, as will be indicated in what follows, have led me to modify in certain respects the conclusions which I first formed regarding the cytological basis of apogamy in this species.

For the past six years I have grown the prothallia of a number of species of ferns for the purpose of determining the occurrence of apogamy and of investigating, so far as possible, the cytology of the apogamous forms. I have reported apogamy in *Pellaea atropurpurea*, L. (Steil, 1910), and later (Steil, 1915 a) in *P. adiantoidis*, J. Sm, *Aspidium chrysoloba*, *A. tsussimense*, and *Nephrodium hirtipes*, Hk. I have also found it to occur in *Pellaea atropurpurea* var. *cristata*, Trel., *P. hastata*, L., *Aspidium falcatum Rockfordianum*, *A. falcatum Fortunei*, *A. falcatum caryotidium*, *Pteris sulcata*, L., *Pteris argyrea*, Moore, *Pteris Parkeri*, and in a number of

varieties of *Pteris cretica* (Steil, 1918). Antheridia are produced on the prothallia of all the species in which I have observed the development of apogamous embryos. Archegonia are, however, developed in only a few of these species. Cytological studies of the apogamous ferns have already been begun, and in several species the history of the sporogenous cells appears to be similar to that herein described for *Nephrodium hirtipes*.

In all the apogamous ferns which have so far been investigated, antheridia appear to be developed except in *Trichomanes alatum* and *T. Kraussii*. In these two species antheridia never develop to maturity. In numerous cultures of the prothallia of *Pellaea atropurpurea*, L., antheridia were found by the writer, but under certain cultural conditions they frequently appear in large numbers. In a number of species archegonia are never produced, in a few they abort, and in still others they are apparently developed to maturity. Whether fertilization occurs in the latter cases, as reported by Heim (1896) and Heilbronn (1910) for some of the apogamous species, is a question that must be left for further investigation.

Materials and Methods.

The reader is referred to my preliminary note on *Nephrodium hirtipes* (1915 b) for a brief description of the cultural conditions under which the prothallia were grown. In the many cultures which have been made since the discovery of apogamy in this species, no important differences have been observed in the development of the prothallia or in that of the apogamously produced embryos.

The spores of *Nephrodium hirtipes* from which the prothallia were grown were collected from plants grown in the university greenhouse. Five of these plants were obtained from the first culture of the prothallia made December 14, 1913. The others were obtained from different sources.

The rate of growth and the size of the prothallia were affected by the strength of the nutrient solution, the temperature, the moisture, and the illumination. Too intense illumination was avoided by shading. The moisture supply was sufficient to allow fertilization to occur in all of the numerous non-apogamous species which have been grown under the same conditions as *Nephrodium hirtipes*. The temperature of the Wardian case in which the prothallia were grown varied from 70° F. in winter to about 110° F. in the hottest days of summer. While a 0·1 per cent. Knop's solution was generally used for the prothallia grown on sphagnum, various strengths of this solution were used and the spores were sown also on the surface of the liquid medium. Beyerinck's solution as modified by Moore (1903) also proved very satisfactory. By tilting the bell-jars which were placed over the uncovered Stender dishes with the growing prothallia, an abundant supply of oxygen and carbon dioxide was provided.

So far as possible an attempt was made to check the influence of the cultural conditions on the development of archegonia and the formation of embryos of apogamous origin. When spores were sown on soil in different parts of the greenhouse the prothallia of *Nephrodium hirtipes* always produced embryos apogamously. Variations in the illumination, the strength of the nutrient solution, or the temperature, resulted neither in the production of archegonia nor in any essential difference in the manner of formation of the embryos. A number of species of ferns in which fertilization is known to occur were also grown in the Wardian case, but they never produced embryos apogamously under these conditions.

Spores of *Asplenium nidus avis* and *Nephrodium molle* obtained through the kindness of Dr. George T. Moore, Director of the Missouri Botanical Garden, were sown, and the resulting prothallia were also subjected to the same cultural conditions under which the prothallia of *Nephrodium hirtipes* were grown. In both species antheridia and archegonia were produced in large numbers and embryos appeared only as a result of fertilization. Therefore, my cultural conditions were not such as to induce apogamy in *Asplenium nidus avis*, in which it was observed by Nagai (1914), or in *Nephrodium molle*, in which induced apogamy was reported by Yamanouchi (1908 c).

In preparing material for cytological study, various fixing agents were used. By far the best preparations were obtained with the Flemming fluids. Flemming's weaker solution gave the best results for the prothallia and the young embryos; the Flemming medium solution proved most satisfactory for work on the sporogenous cells. Sections were cut usually five or six microns in thickness. For some of the studies of the sporogenous cells, sections were made eight or ten microns in thickness. Although a number of stains were used, Flemming's triple stain (safranin, gentian violet, and orange G) gave the best results.

Observations.

The Gametophyte of Nephrodium hirtipes.

The spores of *Nephrodium hirtipes* usually remain capable of germination for about a month after they mature, although some of them retain their germinating power for a much longer period.

The spores germinate within a few days after they are sown. Nothing unusual was observed in the germination of the spores or in the early stages of development of the prothallia. The prothallia become typically heart-shaped (Figs. 1–3, Pl. V). Glandular hairs are produced on both surfaces, especially on the anterior portion and along the margins of the prothallium. Archegonia are never produced, and antheridia are not formed, as a rule, under ordinary cultural conditions. Spermatogenesis has not been studied

in detail, but, so far as I have observed, it seems normal in every respect. The antherozoids are actively motile.

Secondary prothallia were readily induced, and these resembled in all essential respects the primary prothallia, producing, like them, apogamous sporophytes. I have also found that in some other apogamous species, including *Pellaea atropurpurea*, *Pteris cretica albo-lineata*, and *Pteris sulcata*, secondary prothallia produce apogamously sporophytes.

Numerous attempts were made to induce apospory in *Nephrodium hirtipes* by methods which have been described by different investigators to bring about aposporous development of the gametophyte in other ferns, such as placing the leaves of young sporophytes in contact with the soil, attaching to the soil leaves and fronds of older plants, placing portions of leaves on moist soil or sphagnum, and growing prothallia-bearing young embryos in weak light. So far, only the last method has been successful to a very limited degree. Occasionally when prothallia with very young embryos were placed in subdued light, the apical region of the prothallium, frequently almost colourless grew forward into a long cylindrical or conical process, and on the new portion it was observed that after the primary leaf had begun its development as a direct outgrowth of the prothallium, its proximal portion, instead of forming a lamina typical in form and structure, became branched and gradually prothalloid. A root, so far as I could determine, was usually absent in such cases. Fig. 22, Plate V, represents a good specimen of this kind observed in the cultures. The petiole (*p*) was cylindrical and contained a well-developed vascular bundle (*v*), shown only in part in the photograph. No stomata were found on any portion of the sporophytic growth. The cells (*c*) in the flattened portion of the structure next to the terminal part of the petiole were columnar and presented a regular arrangement. There was a gradual change in the form of these cells towards the apical region (*a*) which was already differentiated. A filamentous portion (*f*), consisting of a single row of cells, had been produced from the prothalloid part of the outgrowth. Later an embryo was observed to begin its development directly behind the apical region (*a*). Whether or not such a development of a gametophyte from a sporophyte of this nature is to be called apospory is a question of definition.

The Sporophyte of Nephrodium hirtipes.

Development of the Embryo. The embryo originates from cells posterior to the apical notch of the prothallium and on its ventral side. A light-coloured region frequently appears either in the notch or at some distance behind it (Figs. 4, 5, 6, 10, 11, Pl. V). It is in this light-coloured region that the embryo is usually formed. The cells of this region usually contain numerous colourless plastids, but few chloroplasts as compared with the

neighbouring prothallial cells (Figs. 7 and 8, Pl. V). When prothallia are grown in weak light a larger proportion develop this light region. An early stage in the development of the embryo is shown in Fig. 3, Pl. V. In this case only a few small superficial cells of the embryo are visible on the ventral side of the prothallium. The paler portion, so conspicuous in the prothallia represented by Figs. 4, 10, and 11, Pl. V, is absent in this instance. Sometimes tracheides appear in the portion of the prothallium just described (*a*, Fig. 10, Pl. V), but these are not always present at so early a stage in the development of the embryo. The pale region is generally differentiated before the prothallium has reached its maximum size. The growth of the prothallium continues for some time even after the apical cell of the primary leaf and that of the primary root have been differentiated.

From numerous sections made of prothallia showing early stages in the development of embryos, it has not yet been determined whether a single superficial cell only is concerned in the formation of the embryo as described by Yamanouchi (1908 c) in *Nephrodium molle*. The embryo certainly begins its development very early and even before the cushion of the prothallium has been formed. The details of this development are reserved for future investigation.

The apical cell of the leaf of the embryo is differentiated first, then that of the root, and finally that of the stem. The stem rudiment appears between the base of the leaf and the prothallium. The root is endogenous. A foot, so far as I have been able to determine, is never produced.

As the embryo grows it involves a considerable portion of both the upper and lower parts of the prothallium. This is shown in Figs. 6 (*b* and *c*) and 11, Pl. V. Fig. 12 of the same plate is a dorsal view of the prothallium that is represented in ventral view in Fig. 11. The extent of the tissue belonging to the embryo can be readily determined from the appearance of the cells in a stained section. The cells of the embryo are smaller than the prothalloid cells, and their cytoplasmic contents are much denser. The nuclei of the embryo are large in proportion to the size of the cells, although many appear to be no larger absolutely than the nuclei of the gametophyte. The vascular system which develops in the young embryo grows anteriorly into the leaf and posteriorly towards the root apex. A vascular strand also extends into the anterior portion of the prothallium and in the direction of the apex of the stem.

Multicellular glandular hairs are produced on the young embryo. Sometimes similar hairs are borne also on the light region in the neighbourhood of the notch (Fig. 9, Pl. V).

While the leaf usually appears first in the development of the embryo (Figs. 13, 14, 15), in some cases the root develops before the leaf (Fig. 16, Pl. V). In still other cases the leaf and the root appear simultaneously (Figs.

17–21, Pl. V). Sometimes the leaf is developed from the surface of the prothallium (Fig. 19, Pl. V), and in a few instances it has been found that leaves are developed from both surfaces. Occasionally roots were observed to make their appearance on both surfaces of the prothallium. Secondary prothallia produce embryos in all essential respects similar to those formed by the primary prothallia. Large irregular prothallia whose form results from changes in the intensity of illumination may produce more than one embryo each (Fig. 24, Pl. V, *a* and *b*), and these embryos may develop into normal sporophytes. Fig. 23, Pl. V, represents a portion of a prothallium of irregular form with two embryos, each with a leaf and a root. On another portion of the same prothallium a third embryo had been produced.

A careful study was made to determine whether nuclear migrations and fusions similar to those described by Farmer and Digby (1907) occur among the vegetative cells of the prothallium previous to the development of the embryo. For this purpose both stained prothallia mounted whole and stained sections were studied. In not a single instance were nuclear migrations, nuclear fusions, or binucleate cells observed.

Sporogenesis. After it was discovered that in the sporangium of *Nephrodium hirtipes* the chromosome number is doubled in consequence of incomplete nuclear divisions in the manner described below, it appeared desirable to make as thorough a study as possible of the whole subject of sporogenesis. The observations on the sporogenous cells are based on prepared slides of thousands of sporangia in different stages of development. Most of the work was checked by a microscopical examination of living material. The presence of numerous nuclear division figures in the sporogenous cells belonging to the generations previous to the spore mother-cells, in tapetal cells, and in the wall cells of the sporangium, facilitated the counting of the chromosomes. In the dividing nuclei of cells of all these classes between sixty and sixty-five chromosomes were counted. The same approximate number of chromosomes was counted in dividing nuclei of the gametophyte and the young sporophyte.

The nuclear and cell divisions in the sporangium, preceding the stage at which eight sporogenous cells are present, are normal in every respect. At this stage the sporogenous cells are nearly isodiametric, although there are frequent exceptions, some of them being at times much elongated. The resting nucleus is large, although not so large as the new diploid nucleus formed at a later period. The resting nucleus passes through the normal prophase stages. At this time the tapetum is composed of two layers of cells. Normal division figures are not uncommon in the tapetal cells and the sporangial wall cells even, at a later stage.

The metaphases following these prophases are at first normal in appearance, when observed either from the side or in polar view (Fig. 24, Pl. VI). Instead, however, of the metaphases being followed by the

anaphases and telophases in the usual order, a peculiar incomplete nuclear and cell division now occurs.

In the large majority of cases only a number of the daughter chromosomes in the equatorial plate pass to the respective poles of the broad-poled spindle. In other instances the chromosomes appear to pass but a short distance towards the poles. The resting nucleus in the former instance will be either kidney-shaped or dumb-bell-shaped (Figs. 30, 31, Pl. VI), and in the latter spherical (Fig. 26, Pl. VI). It is evident from the foregoing that the new nuclear membrane encloses all the nuclear material which in an ordinary division is distributed to two daughter nuclei. The new nucleus so established thus contains the diploid number of chromosomes.

Fig. 25, Pl. VI, was made from a longitudinal section of a sporangium. Five of the eight sporogenous cells are shown in the section, and in each case some of the chromosomes have passed to the poles of the spindle. In cell *a* all the chromosomes appear to have passed to the poles, but the next section of the same nucleus shows many chromosomes between the two apparently separate groups shown in this figure. Fig. 27 of Pl. VI shows a similar but later stage. Only four of the sporogenous cells are shown in this case. The outlines of the cells are already becoming rounded, which fact indicates that they are to function as spore mother-cells. The chromosomes are beginning to anastomose preparatory to the passing of the nucleus into a resting condition. The nuclear membrane has already been formed and encloses therefore the diploid number of chromosomes. Later stages are represented by Figs. 28, 29, 30, 31, 32, 33, and 34, Pl. VI. In some of the nuclei shown in these figures the nuclear material is beginning to pass into the resting condition. It appears that occasionally the nucleus passes into this condition before the nuclear membrane has been formed. It was impossible, however, to obtain any positive evidence on this point. It is not difficult to find in a sporangium of *Nephrodium hirtipes* nuclei of the different forms described in various stages of synapsis and in the heterotypic and homotypic divisions. It is certain that the cells with these nuclei function as spore mother-cells.

During synapsis a large number of the nuclei of the spore mother-cells are spherical in form. The number of nuclei of the kidney-shaped and the dumb-bell-shaped types becomes progressively smaller as the spore mother-cells grow older. Unless the incomplete cell divisions to be described later progress too far, there is no reason to doubt that many nuclei, at first irregular in form, later become spherical.

Frequently in the same sporangium spherical nuclei and nuclei of the irregular forms described are present. Often chromatin material in the different nuclei of the sporogenous cells in a sporangium is in the same stage. It appears, from an examination of numerous preparations, that in some instances the chromosomes in the equatorial plate of the eight-celled

stage retain their position in the plate or pass only a short distance towards the poles of the spindle. Fig. 26, Pl. VI, represents a dividing nucleus in which the chromosomes have passed only a short distance towards the poles. The distribution of the chromosomes in this case may suggest a polar view of an equatorial plate stage, but in such a view and at an early stage the chromosomes present a more compact arrangement. Spindle fibres were demonstrated to be present in some cases at this time. From an examination of other sections of the same nucleus, it is probable that neither a kidney-shaped nor a dumb-bell-shaped nucleus will be formed from such a nucleus. Frequently a nearly spherical resting nucleus was found in a sporangium, while adjacent to it was a nucleus of the kidney-shaped type. The spherical nucleus in this case may have resulted from an incomplete nuclear division in which the chromosomes of the equatorial plate passed only a short distance towards the poles.

A number of nuclei in a sporangium become spherical at an early stage, and some sporangia were found in which the nuclei of all eight of the sporogenous cells were spherical, and in this case each nucleus was in the resting condition. Two assumptions can be made in regard to the origin of such nuclei. The resting nuclei of the first eight-celled stage may not divide and function as the nuclei of the spore mother-cells, or they may begin to divide, but the chromosomes may not pass to the poles. From what has been stated in regard to the history of the chromosomes in the equatorial plate, it is very probable that the latter assumption is true. In some sporangia, as previously stated, all the nuclei are either kidney-shaped or dumb-bell-shaped, while in other sporangia the nuclei vary from the spherical to the kidney or dumb-bell forms. It would not, therefore, be surprising to find a sporangium all of whose nuclei were spherical in form, even though an incomplete division had occurred in each.

Spindle fibres were in some instances stained with difficulty, and at first were overlooked. These fibres are shown in Figs. 24, 25, 27, 28, 29, of Pl. VI. In the cell shown in Fig. 28 a cell plate was already formed although no nuclear membrane was present. Sometimes the fibres are well developed, but there is no indication of a cell plate (Figs. 25 and 27, Pl. VI). As the different longitudinal sections of nuclei indicate, spindle fibres are never present on the convex side of the nucleus. In a number of instances spindle fibres appeared to be absent even on the concave side of the nucleus. In some cases, on account of the distribution of the fibres, the plate formed on the spindle did not extend to the nuclear membrane (*c*, Fig. 27, Pl. VI). In the majority of cases, however, the plate extends from the wall of the cell to the nuclear membrane (Figs. 29–33, Pl. VI). In some instances the fibres are poorly developed or quite absent. This seems to be frequently the case when the nuclei are somewhat irregular in form. When spindle fibres are present a cell plate is often produced and a distinct cell wall is formed later,

extending part way across the cell. Hence when a new wall is produced it extends from the wall of the cell to the concavity of the nucleus (Figs. 30, 31, 32, and 33, Pl. VI).

Fig. 32, Pl. VI. presents some evidence that if spindle fibres are formed during the division of the nucleus they may sometimes wholly or partially disappear before cell-wall formation. On the left-hand side of the figure the new walls are present, but on the other side no indication of such walls is observed. Fig. 33 was drawn from the same sporangium. Such figures are very infrequent and suggest that complete nuclear and cell divisions may occur in rare instances. It would be difficult to conceive how Fig. 33 could represent the result of a fusion of two sporogenous cells and their nuclei. The whole figure represents clearly a single cell, and the portions of it have been produced by an incomplete cell division just described. Such a cell could be formed from one similar to *a*, Fig. 25, Pl. VI.

It appears, therefore, that the eight sporogenous cells undergo not only an incomplete nuclear division but also in some instances an incomplete cell division. That nuclear and cell divisions are not completed, except possibly in very rare instances, was determined by counting the sporogenous cells at various stages of development. Many spore mother-cells during the resting stage of the nucleus, synapsis, the heterotypic and the homotypic divisions, and spores showed evidences of incomplete division (Figs. 33, 34, Pl. VI, and Figs. 39, 43, 49, 50, 51, and 52, Pl. VII). Hence there can be no question that cell and nuclear divisions rarely, if ever, occur during the first eight-celled stage of the sporogenous cells.

Except in the shape of the cell or its nucleus, nothing unusual was observed in the structure or behaviour of the sporogenous cells after the incomplete division just described. The nucleus and the cell increase considerably in size, and unless there is a well-developed spindle produced, and a partial division of the cell occurs, the majority of the cells and their nuclei finally become approximately spherical. A comparison of the sizes of the resting nuclei before and after the incomplete divisions shows that they are considerably larger at the latter stage. While the nuclear material is still in the form of a reticulum, it recedes gradually from one side of the nuclear membrane. A continuous spireme appears to be formed as the nucleus passes into synapsis. Fig. 35, Pl. VI, represents an early stage in this continuous spireme thread. The compact synaptic mass that finally appears pressed against the membrane on one side of the nucleus includes the nucleolus, which seems to persist during the contraction stage (Fig. 36, Pl. VI). When the spireme emerges from synapsis, radiating portions of the thread extend to the nuclear membrane on the side opposite that at which the contraction took place. During the synaptic period the cytoplasmic portion of the mother-cell, where the synaptic mass is present, is very thin. After synapsis the cytoplasm of the mother-cell gradually grows to a nearly

uniform thickness. The mother-cells grow considerably while the foregoing changes are taking place, although they vary much in size at any particular stage. Fig. 39, Pl. VII, is drawn from a flattened spore mother-cell and hence appears unusually large. The details of chromosome formation were not followed. In diakinesis (Figs. 37 and 38, Pl. VI, and Fig. 39, Pl. VII) the chromosomes were counted, and there were found to be present at this time between sixty and sixty-five bivalent chromosomes. A multipolar spindle is formed which becomes bipolar (Figs. 40 and 41, Pl. VII). The various stages in the division of the nuclei of the spore mother-cells were studied, and in the vast majority of cases the divisions are in every way normal (Figs. 42, 43, 44, 45, 46, Pl. VII). Sometimes anaphase figures were found in both the heterotypic and homotypic divisions with lagging chromosomes (Fig. 42, Pl. VII). During the metaphase of the heterotypic division a few of the chromosomes pass to the poles while the remainder are still in the equatorial plate. Between the heterotypic and the homotypic divisions the individual chromosomes can be readily distinguished (Fig. 43, Pl. VII).

During the heterotypic division a granular zone appears in a broad equatorial plate midway between the daughter nuclei. This zone stains heavily and is composed of numerous small granules of cytoplasmic material (Figs. 43, 44, 45, and 46, Pl. VII). In the homotypic division the axis of the spindles may be parallel (Fig. 45, Pl. VII), oblique (Fig. 46, Pl. VII), or perpendicular to one another, or the axes of the two may lie in the same line.

As a result of the division of the spore mother-cells thirty-two spores are produced, each having the reduced number of chromosomes, namely between sixty and sixty-five. Frequently the chromatin material in each member of the young tetrad is aggregated at one side of the nucleus and a clear region appears on the opposite side (Fig. 47, Pl. VII). The cells in the preparation from which the figure was drawn showed no signs of plasmolysis, and since the same appearance was observed when different fixing fluids were used, this condition of the nucleus is not likely to have resulted from the technique employed. There is considerable growth in the size of the spores before the thick wall is formed about each one (Fig. 48, Pl. VII). Sometimes chromatic material is found in the cytoplasm of the young spore (Fig. 54, Pl. VII). The origin of the nuclear material may be traced to chromosomes left in the cytoplasm during the division of the spore mother-cell nucleus.

Since a number of sporogenous cells during the eight-celled stage were found in which the divisions were almost completed, it seemed probable, as I have already suggested, that at least in some cases such divisions may occur. A large number of preparations were examined, but no instance of such a division was found. The cells at different stages were also counted, and in only two cases observed did it appear that more than eight mother-

cells had been formed in a sporangium. In one sporangium three undivided mother-cells and six tetrads were found. There must have been at an early stage, therefore, nine spore mother-cells. One of the eight sporogenous cells which ordinarily undergo the incomplete division already described presumably completed its division in this case, and thus the additional spore mother-cell may have resulted. In one other instance more than the usual number of tetrads was found in a sporangium. In this case there were eleven tetrads, six showing evidences of incomplete divisions, and five of complete divisions. Fig. 53, Pl. VII, represents one of the tetrads with incompletely divided cells. In the plane of the section only one of the cells and its nucleus indicated that the divisions never had been completed. However, the other cell and its nucleus showed similarly that incomplete divisions had occurred. If three of the eight sporogenous cells which as a rule undergo the incomplete divisions described divided, three additional cells would be produced. The other five cells would undergo the usual divisions in the formation of the tetrads; but six of the cells, it may be supposed, would show abnormalities in the divisions. From such a single instance it is, of course, unsafe to draw any positive conclusions. The two sporangia here described were the only ones which afforded evidence that a complete division of one or more of the eight original sporogenous cells may occasionally occur.

Sometimes, on account of the shape of the spore mother-cell and its nucleus, the chromatin material may be divided during synapsis into two nearly equal masses. In one instance two nearly distinct chromosome groups were observed during diakinesis in a dumb-bell-shaped nucleus. As a result of the usual divisions eight nuclei may be produced. When the spore mother-cells are kidney-shaped or dumb-bell-shaped, the chromosomes during the anaphase of the heterotypic division at one or both of the poles may occasionally be separated into two groups (Fig. 49, Pl. VII). The separation of the chromosomes in this instance is clearly brought about by the indentation of the mother-cell resulting from the original incomplete division. Each of the three nuclei represented in this figure may divide (Fig. 50, Pl. VII) and six spores may be formed. Fig. 52, Pl. VII, shows five of the six nuclei which result in such a case. Several metaphase figures were found in the preparations similar to that shown in Fig. 50, but only one spore mother-cell containing three anaphase figures was observed (Fig. 51, Pl. VII). That the two smaller division figures represented in the figure resulted from a division of one of two groups of chromosomes produced during the anaphase of the heterotypic division cannot be stated with certainty. When there are two indentations in the spore mother-cell there might conceivably be produced during the anaphase of the heterotypic division four chromosome groups, and in consequence of the following division eight spores might be formed from a single spore mother-cell. No

such instances were observed in any of my preparations. Whether such spores whose nuclei do not possess the normal number of chromosomes have the power to germinate has not been determined.

The formation of more than four spores from a single spore mother-cell has been reported in Angiosperms by various investigators. Wille (1886) gives a summary of the early literature of the subject and reports also his own discoveries. Strasburger (1892) and Juel (1897) found that the supernumerary pollen grains of *Hemerocallis fulva* are produced by the formation of cells whose nuclei originate from chromosomes which fail to pass to either pole during the division of the spore mother-cell. Fullmer (1899) observed in the same species that occasionally some of the nuclei of the spore-tetrads divide, and that as a result of these divisions additional microspores are produced. Miss Lyon (1898) reported that in *Euphorbia corollata* sometimes five or six microspores are produced in place of the usual four spores of a tetrad. No explanation is given for the presence of the supernumerary spores in this plant. So far as I know, the formation of more than four spores from a spore mother-cell has not previously been described in any of the Bryophytes or Pteridophytes.

Discussion.

In my preliminary note (1915 b) I described cell and nuclear fusions in the sporangia of *Nephrodium hirtipes* similar to those described by Miss Allen (1911) in *Aspidium falcatum*. Since sporangia of *Nephrodium hirtipes* were found containing eight sporogenous cells whose nuclei in some cases were in prophases and in other cases in metaphases, it seemed that the completion of the divisions must result in the formation of sixteen sporogenous cells in each sporangium. In older sporangia figures were found which seemed to show that these sixteen cells were fusing in pairs. The presence of thirty-two spores in each sporangium, evidently produced by the division of eight spore mother-cells, apparently confirmed this conclusion. However, a later and more extended study of additional preparations, which contained an abundance of division figures, has shown that the divisions first initiated at the stage of the eight sporogenous cells are never or very rarely completed; that these incomplete divisions result in irregular cell and nuclear forms, as well as in the partial division of the cells by cell-plates or walls, which were first interpreted as evidences of cell fusion; and that the result of these incomplete divisions, as indicated in what precedes, is a doubling of the number of chromosomes in each of the eight cells which then proceed to function as spore mother-cells. A later examination of my older preparations shows, as a matter of fact, that they contain very few earlier stages in incomplete nuclear and cell division, and hence the most important evidence on the history of the sporogenous cells was not at first obtained.

Miss Allen's figures of the sporogenous cells of *Aspidium falcatum* are so similar to many of those which I have obtained in *Nephrodium hirtipes* as to make it seem probable that a further study of the former species may show that in it also the divisions first initiated at the stage of the eight sporogenous cells are, except in very rare cases, never completed. Her Fig. 44, Pl. III, representing an early stage in fusion, may be interpreted as showing a completed nuclear division and a nearly completed cell division, and if this be a correct interpretation the division shown in this case has progressed farther than any I have observed in *Nephrodium*. However, in some instances, as in Fig. 33, Pl. VI, my preparations show a near approach to a complete division of the cell and its nucleus.

In three cases Miss Allen found more than eight spore mother-cells in a sporangium of *Aspidium falcatum*. The larger number of spores was accounted for by the failure of some of the sporogenous cells to fuse in pairs. In two instances I found that more than eight sporogenous cells must have been produced in a sporangium of *Nephrodium hirtipes*. If in these instances any of the sporogenous cells complete their divisions, more than eight spore mother-cells will be formed. No evidence has been presented by either Miss Allen or myself to indicate that the spore mother-cells which do not possess the diploid number of chromosomes have the power to divide. Six of the eleven tetrads found in a single sporangium of *Nephrodium* (Fig. 53, Pl. VII) showed that abnormalities had occurred in the division of the mother-cells, which, as has been previously suggested, may have been produced from sporogenous cells that completed their divisions and hence did not have the diploid number of chromosomes. Since more than four spores are sometimes produced from a single spore mother-cell of *Nephrodium hirtipes*, no conclusion can be drawn from the number of spores in a sporangium in regard to the possibility of the division of the supernumerary spore mother-cells. It is evident, however, that spores may be formed with nuclei which have not received the haploid number of chromosomes (Figs. 50, 51, and 52, Pl. VII).

The formation in many instances of a spindle, and not seldom of a cell plate extending part way across the cell, and the distribution of the chromosomes from the equatorial plate exclude the possibility that the irregular nuclear figures which I have found in the eight sporogenous cells of *Nephrodium hirtipes* can in any case represent amitotic division. Amitosis, also, cannot occur at any later stage, since in such a case more than thirty-two spores would then be produced.

In apogamous ferns three cases have been reported in which 'substitution fusions' are thought to occur—that is, fusions of cells (or nuclei) other than those which are morphologically gametes, by means of which the diploid number of chromosomes is established. In the two *Lastraea* varieties studied by Farmer and Digby (1907), in which the fusion is between nuclei

of two vegetative cells of the prothallium, the haploid number of chromosomes characterizes the gametophyte, the diploid number the sporophyte. In *Aspidium falcatum*, in which according to Miss Allen (1911) the fusion is between sporogenous cells, chromosome reduction immediately following, the haploid number of chromosomes is characteristic of both generations. So far as the chromosome number is concerned the same condition prevails in *Nephrodium hirtipes* as in *Aspidium falcatum*.

The chromosome number in other apogamous ferns, so far investigated, is of interest. As a result of induced apogamy in *Nephrodium molle*, according to Yamanouchi (1908 a, b, and c) the haploid number is found in both the gametophyte and the sporophyte. Farmer and Digby (1907) think that in *Lastraea pseudo-mas* var. *cristata apospora* both generations possess the haploid number of chromosomes. On the other hand, the same investigators found that in *Athyrium Filix-foemina* var. *clarissima*, Jones, the diploid number of chromosomes persists throughout both generations. Thus it appears from the studies so far made of apogamous ferns, as well as by evidence from other groups of plants, that the characteristic differences between gametophyte and sporophyte are not determined alone by differences in the chromosome number, but that the sharply contrasted developmental possibilities of spore and zygote are governed by other factors which are as yet entirely unknown.

Apogamy in ferns developed in all probability at a late period in the evolution of the homosporous ferns. So far no certain case of apogamy has been discovered in the Eusporangiatae. Jeffrey (1896), however, believed that he found in one instance evidence of apogamy in *Botrychium virginianum*. A large number have been found among the Polypodiaceae, the most highly specialized, and probably the most recent, homosporous leptosporangiate family.

In regard to the origin of apogamy in *Nephrodium hirtipes* three possibilities are presented. If at one time in the life-history of the fern fertilization was by some means suppressed, the archegonia may have disappeared and fertilization have thus been rendered impossible. The embryo may then have arisen as a vegetative outgrowth. As a substitution for fertilization the incomplete divisions described in this paper may have occurred. Why a doubling of the number of chromosomes should still be necessary for the maintenance of the life-cycle is difficult to explain, since, as has just been stated, in *Lastraea pseudo-mas* var. *cristata apospora* the haploid number of chromosomes remains unchanged throughout both generations, and although Yamanouchi (1908) did not investigate sporogenesis in the apogamously produced sporophytes of *Nephrodium molle*, it is probable that the gametophyte number is maintained in both generations of the fern.

On the other hand, the occurrence of incomplete nuclear divisions

in the sporangium of *Nephrodium hirtipes* may have preceded the appearance of apogamy. If the number of chromosomes was not at first reduced, the act of fertilization was rendered unnecessary, and hence the embryo may have arisen at first parthenogenetically and later apogamously. If the latter view is held, the double number of chromosomes would be present throughout the life-history. However, on the basis of other investigations of different apogamous species, it is probable that the chromosome number now characteristic of both generations of *Nephrodium hirtipes* is the original haploid number, and hence it seems likely that apogamy may have arisen before the incomplete nuclear divisions were established. These views regarding the origin of apogamy are similar to those expressed by Miss Allen (1911) with reference to *Aspidium falcatum*.

Although so far no cytological evidence has been presented to confirm the occurrence of hybridization in ferns, it is possible that *Nephrodium hirtipes* and perhaps some other apogamous ferns may be of hybrid origin, and that the abnormalities which I have described have resulted from fertilization between two more or less closely related species.

Summary.

1. The prothallium of *Nephrodium hirtipes* is produced by the germination of a spore.

2. The gametophyte never produces archegonia, but antheridia are formed which develop apparently normal antherozoids.

3. The development of secondary prothallia is readily induced by cultural conditions.

4. Attempts to induce an aposporous gametophyte development in this species have been successful only in rare instances.

5. The embryo originates at an early stage in the development of the gametophyte as a vegetative outgrowth of the prothallium. The apical cell of the leaf is first formed, and then that of the root, and later that of the stem. A foot is never produced. The later stages in the development of the embryo resemble those of the ordinary fern embryo produced as a result of fertilization.

6. At no time have nuclear migrations and fusions been observed to occur in the prothallium when the embryo begins its development.

7. At the stage when the sporangium contains eight sporogenous cells, an incomplete nuclear and cell division occurs in each of these eight cells. As a result of the incomplete divisions, each nucleus contains the diploid number of chromosomes—between 120 and 130. The eight sporogenous cells, now diploid, function as spore mother-cells. The spores formed from these cells have the haploid number of chromosomes—between 60 and 65—and this number is retained in the cells of both the gametophyte and of the apogamously developed sporophyte.

8. Thirty-two spores are ordinarily produced in a sporangium of *Nephrodium hirtipes*. The smaller number of spores which sometimes occur is to be accounted for by abnormalities in the nuclear condition of the spore mother-cell.

9. A larger number than thirty-two spores is sometimes produced by the formation of more than four spores from a single spore mother-cell. In rare instances more than eight spore mother-cells appear to be produced in a sporangium. Whether in such a case all the spore mother-cells can form normal tetrads and spores has not been determined.

To Professor C. E. Allen I wish to express my thanks for helpful suggestions and criticisms received during the progress of the foregoing investigation.

Bibliography.

Abbot, James (1874): Scalariform Ducts in Prothallia. Quart. Journ. Mic. Sci., new ser., xiv. 401–2.

Allen, R. F. (1911): Studies in Spermatogenesis and Apogamy in Ferns. Trans. Wis. Acad. Sci., Arts, and Letters, xvii. 1–44.

Berggren, S. (1888): Ueber Apogamie von *Nothochlaena*. Bot. Cent., xxxv. 183–4.

Black, Caroline A. (1909): The Development of the Imbedded Antheridium in *Dryopteris stipularis* (Willd.), Maxon, and *Nephrodium molle*. Bull. Tor. Bot. Club, xxxvi. 557–72.

Bower, F. O. (1888): On Some Normal and Abnormal Developments of the Oophyte of *Trichomanes* Ann. Bot., i. 269–305.

de Bary, A. (1878): Ueber apogame Farne und die Erscheinung der Apogamie im allgemeinen. Bot. Zeit., xxxvi. 449–87.

Digby, L. (1905): Preliminary Note on Apospory. Proc. Roy. Soc., 76 B, 463–8.

Farlow, W. G. (1874): An Asexual Growth from the Prothallium of *Pteris cretica*. Quart. Journ. Mic. Sci., xiv. 266–72.

Farmer, J. B., and Digby, L. (1907): Studies in Apospory and Apogamy in Ferns. Ann. Bot., xxi. 161–99.

Farmer, J. B., Moore, J. E. S., and Digby, L. (1903): On the Cytology of Apogamy and Apospory. I. Preliminary Note on Apogamy. Proc. Roy. Soc., lxxi. 453–8.

Fullmer, E. L. (1899): The Development of the Microsporangia and Microspores of *Hemerocallis fulva*. Bot. Gaz., xxviii. 81–8.

Goebel, K. (1905): Aposporie bei *Asplenium dimorphum*. Flora, xcv. 239–44.

Heim, Carl (1896): Untersuchungen über Farnprothallien. Flora, lxxxii. 329–73.

Heilbronn, A. (1910): Apogamie, Bastardierung und Erblichkeitsverhältnisse bei einigen Farnen. Flora, ci. 1–42.

Jeffrey, E. C. (1896): The Gametophyte of *Botrychium virginianum*. University of Toronto Studies, Biol. Ser. No. 1, pp. 1–32, Pl. 1–4.

Juel, H. O. (1897): Die Kerntheilungen in den Pollenmutterzellen von *Hemerocallis fulva* und die bei denselben auftretenden Unregelmässigkeiten. Jahrb. für wiss. Bot., xxx. 205–26.

Lang, W. H. (1898): On Apogamy and the Development of Sporangia from Fern Prothallia. Phil. Trans. Roy. Soc., London, cxc B, 187–239.

Leitgeb, H. (1885): Die Sprossbildung an apogamen Farnprothallien. Ber. Deut. Bot. Ges., iii. 169–76.

LESZCYC-SUMINSKI, J. (1848): Zur Entwickelungsgeschichte der Farrnkräuter. Berlin.
LYON, FLORENCE M. (1898): A Contribution to the Life-history of *Euphorbia corollata*. Bot. Gaz., xxv. 418–26.
MERCKLIN, C. E. (1850): Beobachtungen a. d. Prothallium der Farrnkräuter. St. Petersburg.
MOORE, G. T. (1903): Methods for growing Pure Cultures of Algae. Jour. Appl. Microscopy, vi. 2309–14.
MOTTIER, D. M. (1915): Beobachtungen über einige Farnprothallien mit bezug auf eingebettete Antheridien und Apogamie. Jahrb. für wiss. Bot., lvi. 65–84.
NAGAI, ISUBURO (1914): Physiologische Untersuchungen über Farnprothallien. Flora, cvi. 281–330.
PACE, LULA (1913): Some Peculiar Fern Prothallia. Bot. Gaz., l. 49–58.
SADEBECK, R. (1879): Schenk, Handbuch der Botanik, i. 231–5.
STANGE, F. T. (1887): Mittheilung über seine Farnkulturen und die bei denselben beobachtete Apogamie. Sitz. Bot. Ges., Hamburg, 1886. Also Just's Bot. Jahresber., cli. 598.
STEIL, W. N. (1910): Apogamy in *Pellaea atropurpurea*. Bot. Gaz., xlii. 400–1.
——— (1915 a): Some New Cases of Apogamy in Ferns. Prel. Note. Sci., N.S., xli. 293–4.
——— (1915 b): Apogamy in *Nephrodium hirtipes*. Bot. Gaz., lix. 254–5.
——— (1918): Studies of some New Cases of Apogamy in Ferns. Bull. Torr. Club, 45, pp. 93–108, Pl. 4–5.
STEPHENS, E. L., and SYKES, M. G. (1910): Preliminary Note on Apogamy in *Pteris droogmantiana*. Ann. Bot., xxiv. 487.
STRASBURGER, E. (1892): Ueber den Theilungsvorgang der Zellkerne und das Verhältniss der Kerntheilung zur Zelltheilung. Archiv Mikr. Anat., xxi. 476–590.
WIGAND, A. (1849): Zur Entwickelungsgeschichte der Farnkräuter. Bot. Ztg., vii. 105–7.
WILLE, N. (1886): Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen und das Wachsthum der Membranen durch Intussusception. Christiania Vidensk. Selskabs Forh., 1886.
WORONIN, H. (1907): Apogamie und Aposporie bei einigen Farnen. Ber. Deut. Bot. Ges., xxv. 85–6.
——— (1908): Apogamie und Aposporie bei einigen Farnen. Flora, xcviii. 101–62.
YAMANOUCHI, S. (1908 a): Spermatogenesis, Oogenesis, and Fertilization in *Nephrodium*. Bot. Gaz., xlv. 145–75.
——— (1908 b): Sporogenesis in *Nephrodium*. Ibid., 1–30.
——— (1908 c): Apogamy in *Nephrodium*. Ibid., 289–319.

EXPLANATION OF PLATES V–VII.

Illustrating Dr. W. N. Steil's paper on Apogamy in *Nephrodium hirtipes*.

The photo-micrographs on Plate V represent different magnifications. The young sporophytes from which photographs 13–21 and 23 were made are magnified about 2½ diameters. The figures on Plates VI and VII, excepting 29, 30, and 31, were drawn with a Zeiss compensating ocular No. 12, and apochromatic objective, 2 mm. N. A. 1.30, at table level with the aid of a camera lucida. With a tube elongation of 142 mm. a magnification of about 2,150 diameters was obtained. Figs. 29, 30, and 31 represent a magnification of about 1,250.

PLATE V.

Fig. 1. A dorsal view of a young prothallium.

Fig. 2. A dorsal view of a young prothallium just before the embryo becomes visible.

Fig. 3. An older prothallium, viewed from the ventral side. The small dark region posterior to the apical notch represents the outer cells belonging to the young embryo.

Fig. 4. A dorsal view of a highly magnified portion of a prothallium. The embryo is visible as a compact area in the paler portion.

Fig. 5. A dorsal view of an older embryo.

Fig. 6. Dorsal views of three prothallia at different stages of development. Paler portion of the prothallium produced before the embryo appears shown in *a*. In both *b* and *c* light areas and young embryos shown.

Fig. 7. A highly magnified view of a small portion of a 'light' region.

Fig. 8. A highly magnified view of a small portion in the neighbourhood of the light region.

Fig. 9. A highly magnified portion of the apical region of a prothallium after the embryo has begun its development. *h*, multicellular hair.

Fig. 10. A portion of a prothallium showing the apical region and tracheides, *t*, in paler portion.

Fig. 11. A highly magnified view of a prothallium, dorsal view. *h*, multicellular hair produced on young embryo.

Fig. 12. Ventral view of the same prothallium.

Figs. 13, 14, 15. Young embryos with leaves developed in advance of the roots.

Fig. 16. An embryo with a root produced in advance of the leaf.

Fig. 17. Embryo with leaf developed on the dorsal side of the prothallium.

Fig. 18. An embryo whose root and leaf have grown to about the same length.

Figs. 19, 20. Young embryos. Leaves in advance of the roots.

Fig. 21. An embryo with three leaves.

Fig. 22. A prothallium obtained from a young embryo when a culture was placed in weak illumination. *p*, petiole-like portion of a leaf; *c*, flattened outgrowth with elongated cells; *a*, apical region of gametophytic portion; *f*, filament.

Fig. 23. A prothallium on which three apogamously produced embryos were formed. Two of these are shown in the photograph.

PLATE VI.

Fig. 24. Equatorial plate of the eight-celled stage of the sporogenous cells.

Fig. 25. Five of the eight sporogenous cells drawn from a longitudinal section of a sporangium. In the dividing nuclei some of the chromosomes have passed to the poles. Spindle fibres present.

Fig. 26. A dividing nucleus of one of the eight cells. The chromosomes have not passed to the poles.

Fig. 27. Four of the eight sporogenous cells drawn from a longitudinal section. The chromosomes are beginning to lose their identity *c*, clear region where no spindle fibres are present; *a*, nuclear membrane encloses in each cell the diploid number of chromosomes.

Fig. 28. Some of the chromosomes have passed to the poles. Spindle fibres and cell-plate present.

Fig. 29. A later stage. Spindle fibres and cell-plate shown on the concave side of the nucleus.

Fig. 30. A kidney-shaped nucleus. A wall has been produced on the concave side of the nucleus.

Fig. 31. A dumb-bell-shaped nucleus. Wall shown extending to the concavity of the nucleus.

Fig. 32. A dumb-bell-shaped nucleus. Cell division has progressed still farther than in the preceding stage. Cytoplasm on one side of the nucleus divided into two distinct portions, and a partial separation of the latter has resulted.

Fig. 33. A dumb-bell-shaped nucleus. There was produced in this case a nearly complete nuclear and cell division.

Fig. 34. A kidney-shaped nucleus. The cell is beginning to round up. The indentation on the left-hand side of the figure is the result of incomplete cell division.

Fig. 35. A spore mother-cell whose nucleus is preparing for synapsis.

Fig. 36. The nucleus of the spore mother-cell just emerging from synapsis.

Figs. 37 and 38. The chromosomes during diakinesis. Fig. 37 represents an early stage.

PLATE VII.

Fig. 39. A much flattened spore mother-cell at the same stage as represented in Fig. 15. Indentation shown on one side as a result of incomplete cell division.

Fig. 40. The heterotypic division. Spindle, multipolar.

Fig. 41. Equatorial plate stage of the heterotypic division. Spindle bipolar.

Fig. 42. An anaphase of the heterotypic division. Some lagging chromosomes shown.

Fig. 43. A telophase stage of the heterotypic division. Granular zone present.

Fig. 44. Metaphases of the homotypic division. Spindle multipolar.

Figs. 45 and 46. Anaphase stages of the homotypic division.

Fig. 47. A young tetrad. A clear region on one side of the nucleus.

Fig. 48. An older tetrad.

Fig. 49. An anaphase stage of the heterotypic division, showing how one of the two sets of chromosomes may be divided into two groups.

Fig. 50. A metaphase stage following the stage represented by Fig. 26.

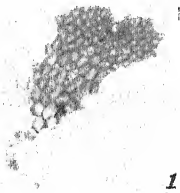
Fig. 51. An anaphase stage.

Fig. 52. A spore mother-cell from which six spores have been produced, five of which are shown in the figure.

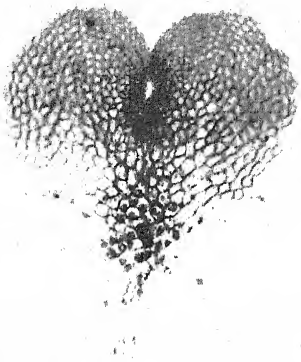
Fig. 53. A tetrad, the members of which have been incompletely divided.

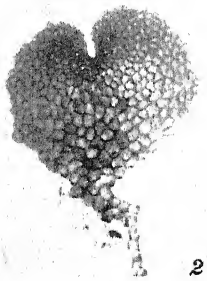
Fig. 54. Two young spores of the same tetrad. Chromatin material in the cytoplasm.

Copyright Wessex Reprints. Not to be reproduced for commercial purposes without permission.

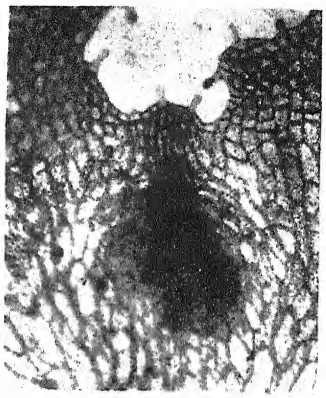


1



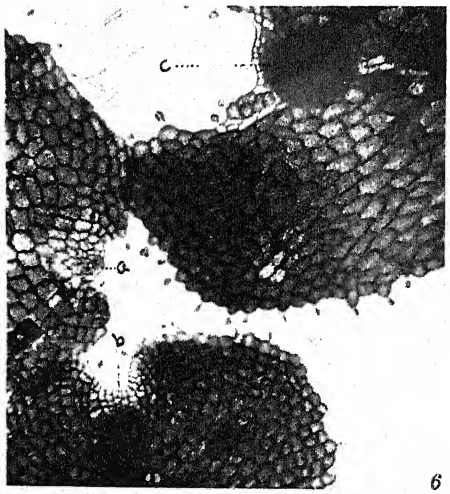


2

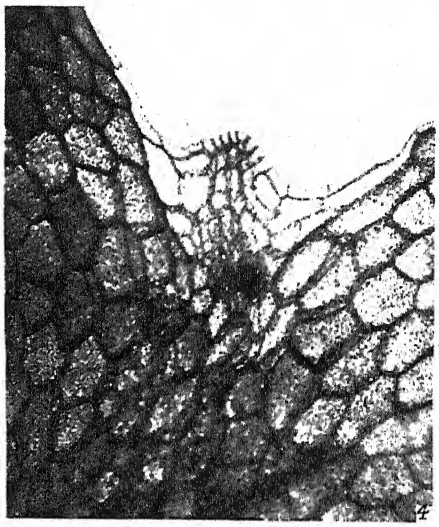


3

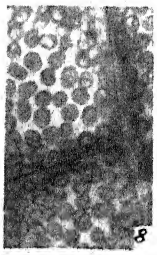
5



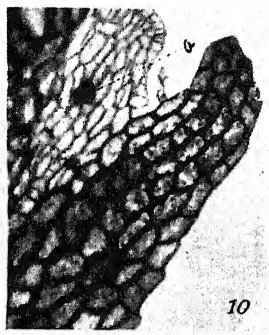
6



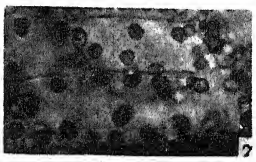
4



8



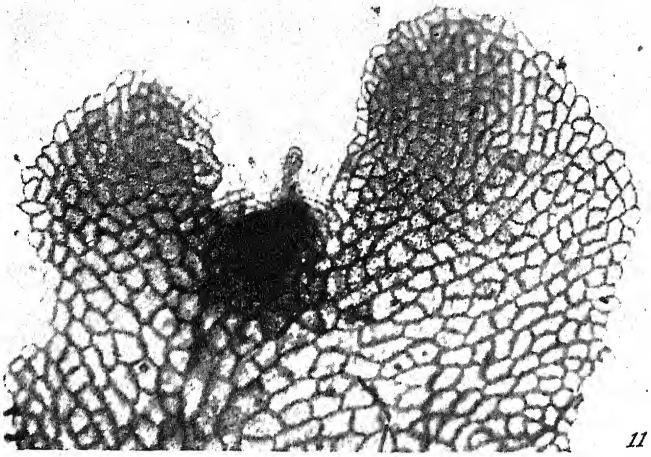
10



7

9

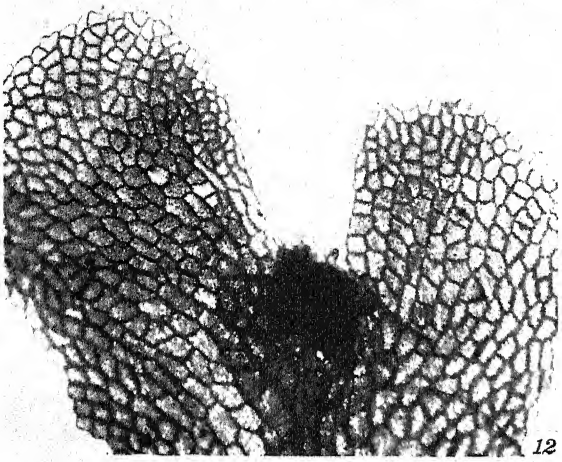
W. N. STEIL— NEPHRODIUM HIRTIPES.



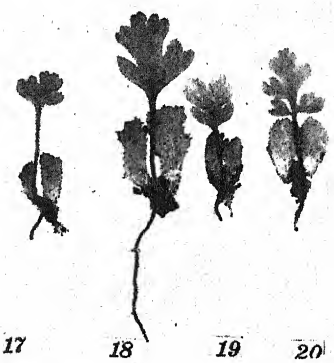
11

13 14

15 16



12

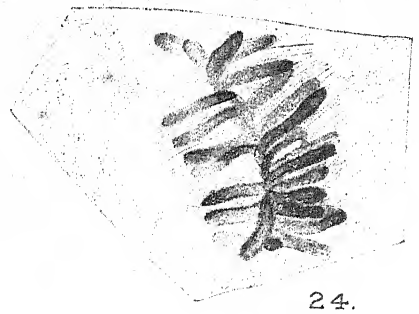


17 18 19 20

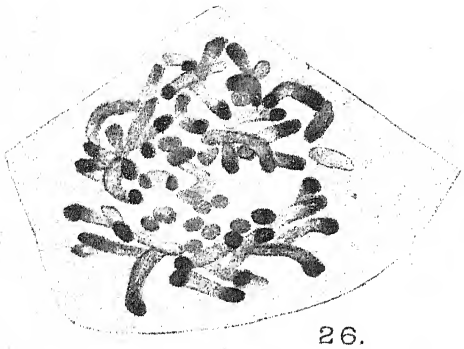
21

23

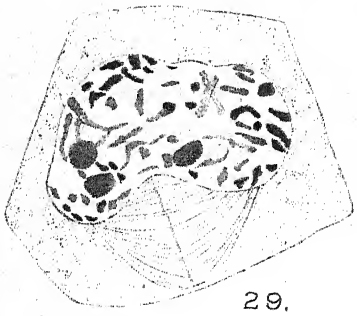
Huth coll.



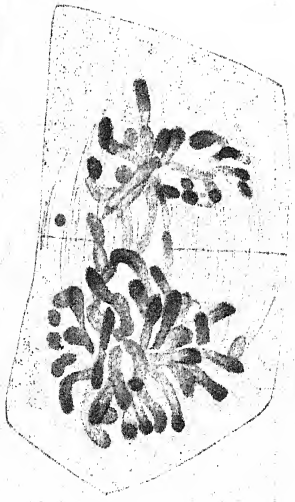
24.



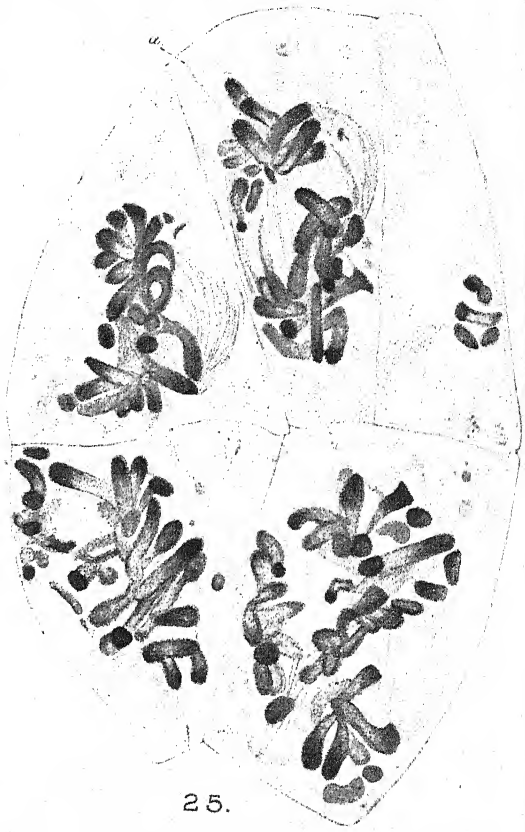
26.



29.



28.



25.

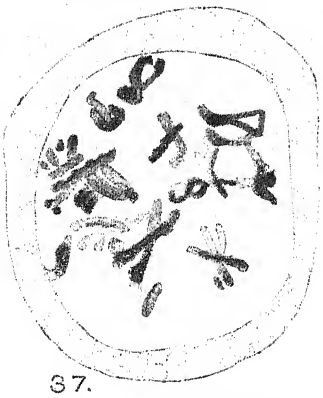
27.

W. N. STEIL — NEPHRODIUM HIRTIPES.

30.

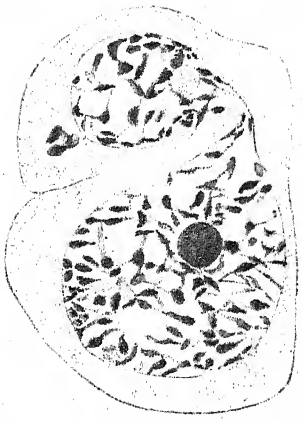
31.

32.

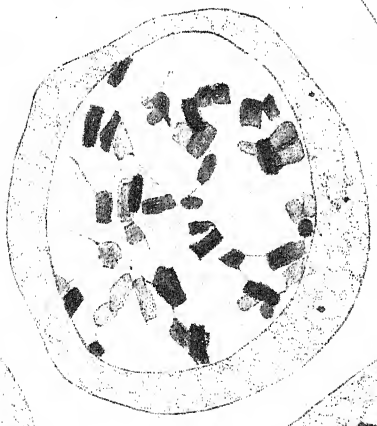


37.

33.

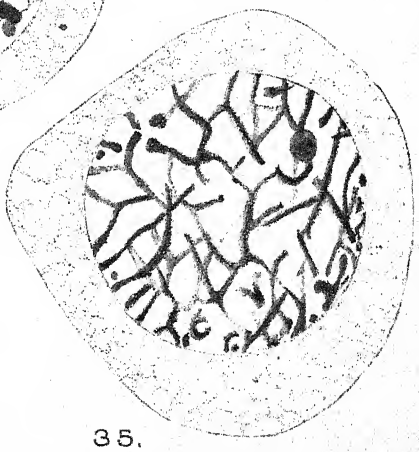


34.



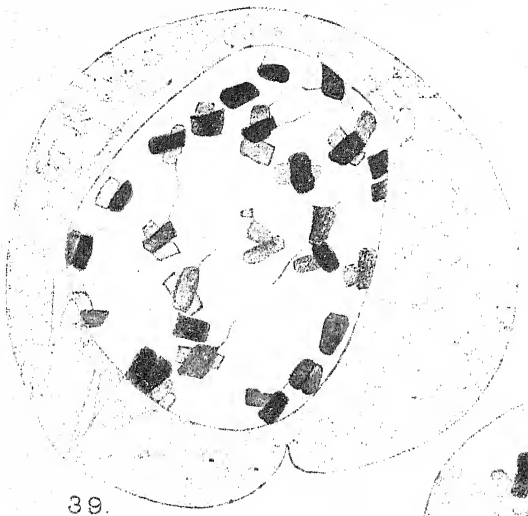
38.

36.

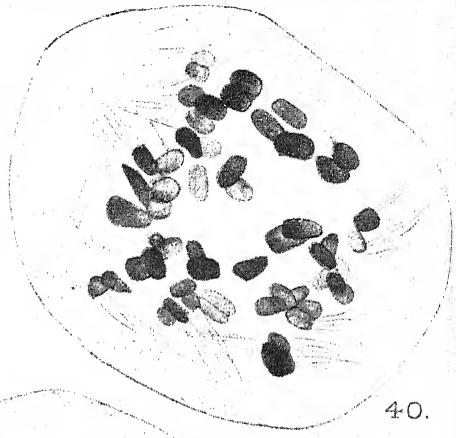


35.

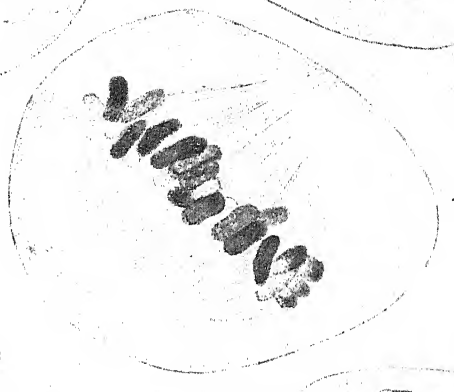
Huth, lith. et imp



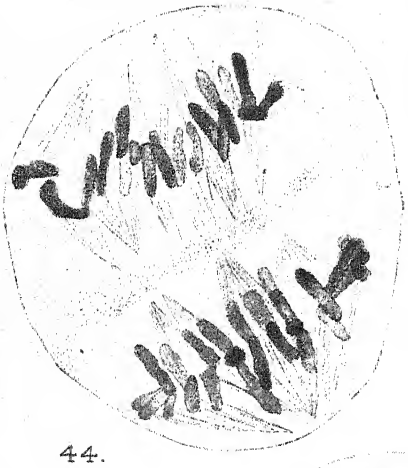
39.



40.



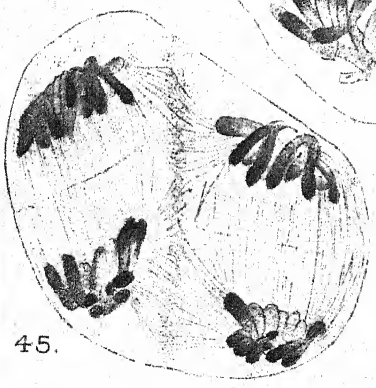
41.



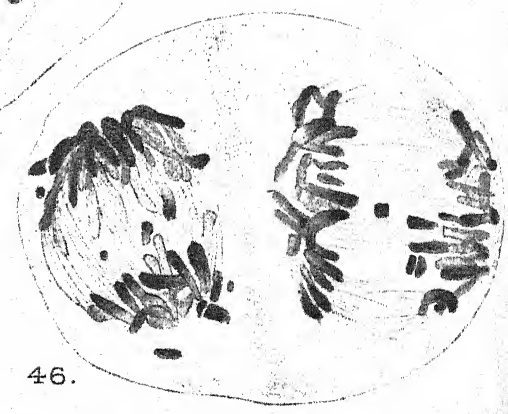
44.

43.

42.

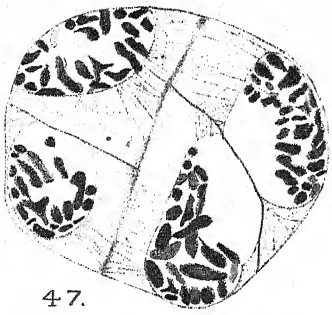


45.

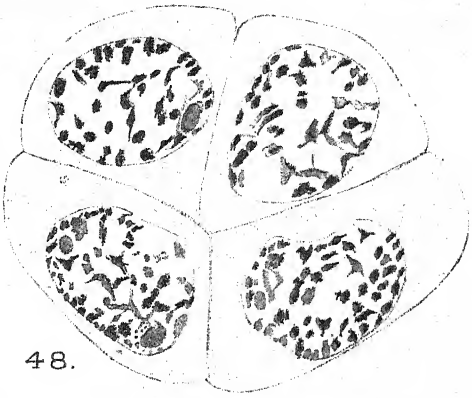


46.

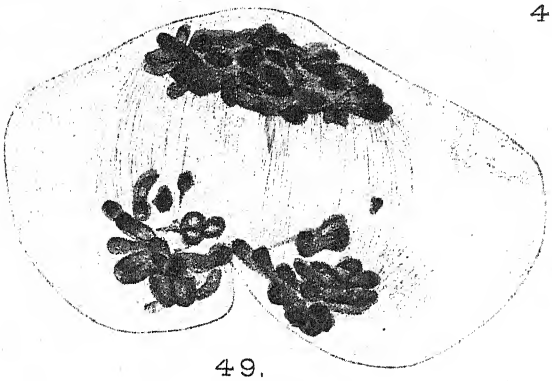
W. N. STEIL—NEPHRODIUM HIRTIPES.



47.

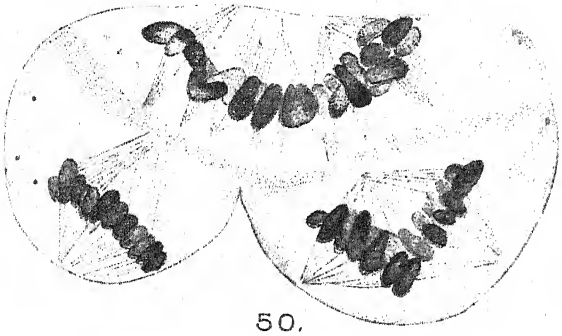


48.

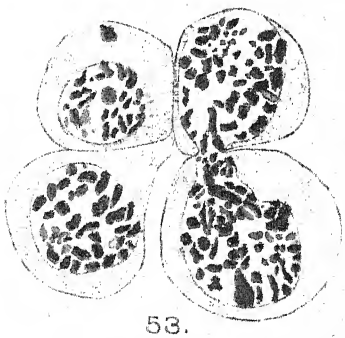


49.

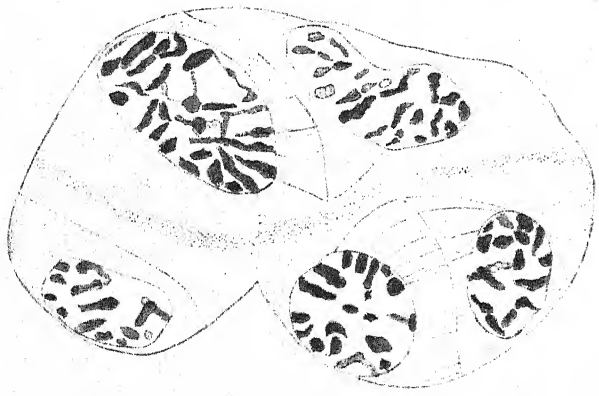
51.



50.



53.



52.

54.

Hanh, lith. et imp.

NOTE

THE WOOD OF TETRACENTRON, TROCHODENDRON, DRIMYS, AND OTHER TYPES.—Messrs. Bailey and Thompson in their paper published in the Annals of Botany, October, 1918, erroneously attribute to me an expression of the view that *Tetracentron* is descended from a form that possessed wood-vessels which have been lost in subsequent evolution. In my paper (Annals of Botany, xxiv, 1910) to which they refer, I merely ventured to suggest and supply evidence in favour of the possibility that the tracheidal structure of the wood of this and certain other genera might represent cases of degeneration from a previous tracheal condition. I then thought, as I still think, that the information available in regard to these anomalous Angiosperms is not sufficient to render possible a decision in favour of either of the two phylogenetic explanations of their wood-structure.

PERCY GROOM.

On the Archesporial and Meiotic Mitoses of Osmunda.

BY

L. DIGBY.

With Plates VIII–XII and one Figure in the Text.

INTRODUCTION.

THE sporogenous tissue of *Osmunda* has been a favourite object for cytological study; not only is it often used for class demonstration, but many cytologists have chosen it for critical examination, and the resulting literature is conflicting in its conclusions. The large size of the nuclei and the remarkably clear and well-defined character of the division figures suggest, on a superficial survey, an easy solution to some of the more complex problems connected with karyokinesis, but, on closer inspection, it will be found that, although the general landmarks of nuclear division are certainly strikingly portrayed, yet factors exist which make the elucidation of the deeper controversial questions exceedingly difficult.

In the first place, the arrangement of the sporangia affords no clue as to the stages of nuclear division in their contained archesporial or spore mother-cells, whereas in many tissues the position of the nuclei is a useful guide in identifying the sequence of the individual phases. For instance, longitudinal sections of anthers generally show the nuclei near the base to be slightly less advanced than those near the apex, and those more centrally placed to be in an intermediate stage; whilst in transverse sections the two outer loculi may be seen to be in advance of the two inner ones, and of each respective pair one loculus may be slightly ahead of the other in the progress of nuclear division. *Osmunda*, on the other hand, gives no such lead, for contiguous sporangia may present widely separated stages. Matters are further complicated owing to the exceeding rapidity with which some of the most critical stages are passed through. Probably others have had a similar experience to that of the writer, and have been baffled year after year in the attempt to elucidate the origin of the heterotype chromosomes owing to the elimination of certain particular phases in the available material which have ultimately proved to be the key to the problem. And lastly, *Osmunda* is difficult to fix satisfactorily. It is simple to achieve a somewhat flashy hard fixation which displays familiar stages well, but it is unsatisfactory for those of crucial importance such as post-synapsis and pre-second contraction.

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

METHODS.

After several seasons of repeated failures, Professor Farmer suggested the simple expedient of immersing the material in warm, weak spirit before placing it in the fixing fluid. This method proved to be a brilliant success, and the ensuing fixation was beautiful, with no apparent contraction or distortion.

Fixing was done exclusively on warm 'growing' days at about noon. Small portions of the sporangiferous apex of the frond were plunged for a few seconds in warm (about 30° C.) 30 per cent. spirit. They were then speedily transferred to fixing fluid at the same temperature. A vacuum pump was attached to the bottle, but the material generally sank immediately without pumping. Rapid infiltration of the fixing fluid is apparently the secret of this almost perfect fixation. Wilson Smith (18) also tried moistening young sporangia in alcohol before placing them in the 'killing' fluid, but, although they sank at once, he reports that the fixation was so poor that they were useless for purposes of study. He does not mention the strength of the alcohol, nor whether it was warmed.

The fixing reagents used in this investigation were strong Flemming, weak Flemming, strong Merkel, Hermann, strong chromic, and acetic alcohol. With acetic alcohol rapid penetration of the fixative occurs without the preliminary alcohol bath, but it is not a sufficiently fine fixative for critical examination. The tissue was left 15 minutes in acetic alcohol and then washed well with methylated spirit, or preferably with absolute alcohol. In the case of the other fixatives, the material was kept in the fluid until the following morning and then washed for four hours in running water. It is most important that the sporangia should not be submitted to any sudden change throughout the subsequent processes to the final embedding in paraffin wax. The material was therefore either put into 10 per cent. glycerine and evaporated slowly to pure, or it was run up by a continuous dropping method devised by Mr. Tabor into absolute alcohol. Cedar-wood oil was found to be the most satisfactory clarifying reagent. After several changes of absolute alcohol, the oil was pipetted into the bottom of the bottle, and the material from floating in a layer of absolute alcohol became gradually impregnated by the oil and sank. After a change into fresh cedar-wood oil the bottle was placed in the embedding oven and shavings of paraffin wax gradually added. In an hour the material was transferred to pure paraffin. Four hours in melted paraffin, with three changes gave excellent results. Most of the sections were cut at 3μ, as it was found that the thinnest sections were the most useful for critical phases.

A variety of stains have been used, but of these Breinl, Flemming's triple, Heidenhain, gentian violet and orange, proved to be the most useful.

This investigation includes several species of *Osmunda* which have been severally minutely examined. The greater number of the drawings have been made from the nuclei of *O. palustris* var. *aurea*, but nuclei of other species have been intercalated when they showed any one particular phase more clearly. The plates therefore comprise figures of *O. palustris*, *O. palustris* var. *aurea*, *O. palustris* var. *undulata*, and *O. regalis*.

The plants have been most successfully grown in greenhouses at the Chelsea Physic Garden under the personal care of Mr. Hales, the Curator, to whom I am greatly obliged.

The following nomenclature will be adopted throughout the paper:

The term '*thread*' will be used to specify the longitudinal *half* of an entire univalent spireme or chromosome which first appears during telophase. The two 'threads' or halves separate during telophase, and reassociate during the ensuing prophase, forming an entire univalent spireme or 'filament' which eventually becomes an entire univalent chromosome. The term '*filament*' will be used to specify the *entire* univalent spireme, the product of the close lateral association of two threads (i.e. of two longitudinal halves of univalent spireme). The term '*strands*' will be used to describe the very fine strands of linin that connect the several chromosome segments of early telophase, also those which serve as fine transverse connexions between the individuals of a pair of associating and dissociating threads, and between the individuals of a pair of conjoining and disjoining filaments.

The terms '*association*' or '*approximation*' will be confined exclusively to the coming together, laterally, in pairs of the 'threads' (i.e. of the two longitudinal halves of the univalent spireme) to form the entire univalent spireme, which becomes the entire univalent chromosome; the terms '*conjunction*' or '*conjoining*' to the coming together in pairs of the 'filaments' (i.e. of the two entire univalent spiremes) to form the bivalent spireme which becomes the bivalent chromosome.

The terms '*fission*' or '*dissociation*' will be restricted to the longitudinal splitting of the 'filament' (i.e. of the entire univalent spireme) into 'threads' (i.e. into half univalent spiremes) and to the splitting of the entire univalent chromosome into daughter chromosomes; the term '*disjunction*' or '*disjoining*' to the separation of the two conjoined 'filaments' (i.e. to the separation of the bivalent spireme into two entire univalent spiremes) and to the separation of the bivalent chromosome into the two entire univalent chromosomes.

Archesporial Divisions.

Probably all cytologists are agreed that the constitution of each individual chromosome is one of duality, and that this dominating feature usually persists throughout the cycles of chromosome construction and dissolution

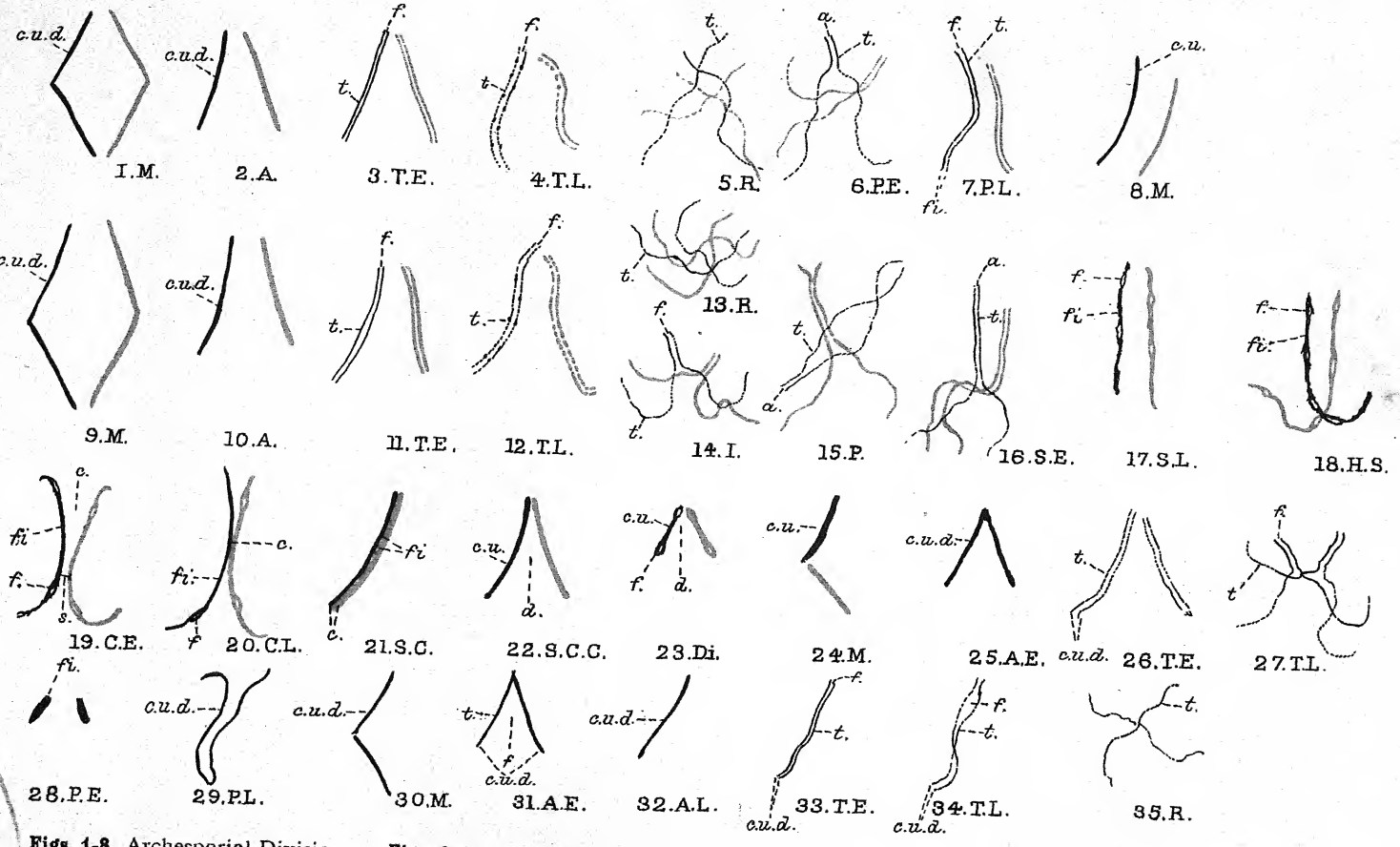
which constitute nuclear mitosis. This double character is apparent more or less continuously, except during the 'rest' which may intervene between successive nuclear divisions, when it may become temporarily obscured. It is represented by paired threads, by paired beads, and by paired segments of spireme. In order that this duality may be clearly appreciated, it is proposed to trace its course briefly before commencing the detailed description of a somatic division (see Text-fig., Nos. 1–8).

If a nuclear division be examined at late anaphase or telophase it will be seen that the individual daughter chromosomes, which have recently separated on the equatorial plate, show fission in varying degrees, thus tending to divide their substance into longitudinal halves (threads). As the newly-formed nuclei prepare to pass into rest the sides or halves (threads) of the chromosomes become beaded, with lengths of linin between the beads. The sides gradually separate from one another and the beads become resolved into ever finer granules, until in complete rest a state of more or less even reticulum is reached. In some plants there is no such thorough chromosome dissolution, and portions of split chromosomes may persist and thus establish visible continuity between anaphase and prophase. During prophase the series of events is reversed, and the chromosome halves (threads) which separated during the preceding telophase approach one another, forming parallel groupings of granules and parallel threads. The space between each pair of associating parallels becomes the future line of fission. When association is consummated in the realization of the completed chromosome, each chromosome opens out and splits into longitudinal halves on the equatorial plate of the spindle, and each half becomes a daughter chromosome. The daughter chromosomes proceed to the poles, and thus the cycle is completed.

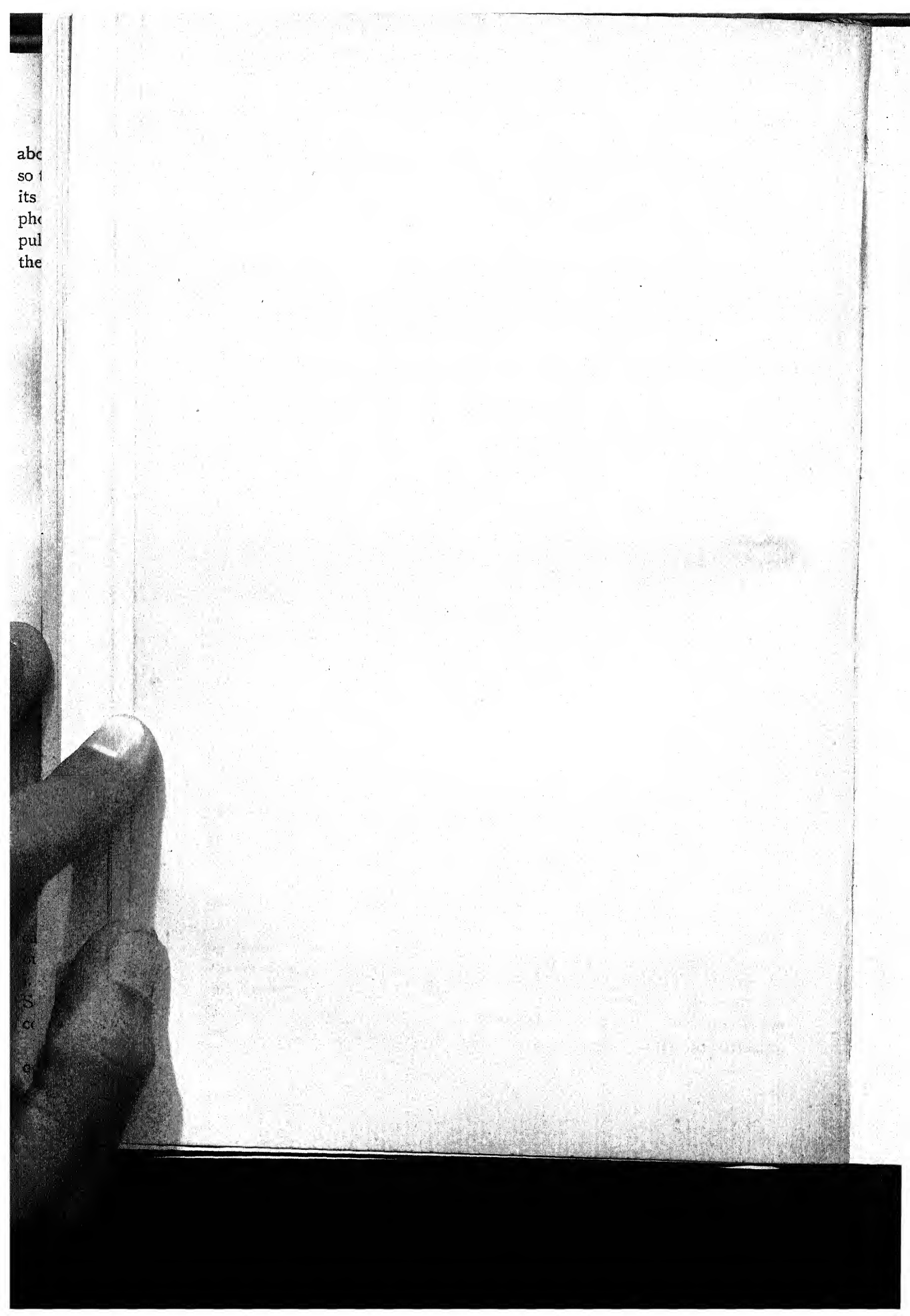
Such is the scheme of a typical somatic mitosis, and it will be shown later how this simple procedure is elaborated in the heterotype division. Occasionally, as in *Primula* (4), a modification occurs, owing to the longitudinal halves (threads) of the chromosomes tending to hold together, instead of to separate. Accordingly, the entire chromosome in telophase inclines to break up transversely into beads or segments, instead of to split into two parallel threads. When the nucleus enters upon prophase, the chromosomes are reorganized by the entire univalent segments coming together again end to end like a string of beads.

The archesporium has been selected for the study of vegetative divisions, and the description of the cycle of phases passed through by a nucleus will commence with the anaphase. This is a clearly defined stage with the daughter chromosomes newly arrived at the poles, and thus affords a good starting-point whence to trace the dissolution and subsequent reconstruction of the chromosomes.

The daughter chromosomes, having arrived at the spindle poles, at first



Figs. 1-8, Archesporial Divisions. **Figs. 9-12,** Last Archesporial Division from Metaphase. **Fig. 13,** Rest between Last Archesporial Division and Heterotype Prophase. **Fig. 14,** Intermediate Stage between the Telophase of the Last Archesporial Division and the Heterotype Prophase. **Figs. 15-27,** Heterotype Division. **Figs. 28-34,** Homotype Division. **Fig. 35,** Resting stage of Tetrad.
A=anaphase, A.E=dº early, A.L=dº late, C.E=conjunction early, C.L=dº late, Di=diakinesis, H.S=hollow spireme, I=stage between telophase of last archesporial division and prophase of heterotype, M=metaphase, P=prophase, P.E=dº early, P.L. dº late, R=rest, S.C=second contraction, S.C.C.=dº coming out of, S.E.=synapsis early, S.L=dº late, T.E.=telophase early, T.L.=dº late.
a=association, *c*=conjunction, *c.u.*=univalent chromosome, *c.u.d.*=dº daughter, *d.*=disjunction, *f*=fission, *fi.*=filament, *s*=strand, *t.*=thread.



show little or no sign of fission (Pl. VIII, Fig. 1); but before the nuclear limiting membrane makes its appearance, and whilst the chromosomes, as seen from a polar view, are still arranged in a rosette, they show marked fission throughout their substance (Fig. 2). Sometimes the sides (threads) are homogeneous, sometimes they are beaded. By the time that the nucleus is bounded by a membrane some of the chromosomes have begun to lose their visible identity; the split widens, and the chromatin tends to separate out along the fine connecting strands that join the several chromosome segments (Fig. 3). When the chromosome halves (threads) are practically resolved into small beads, the skeleton of each quondam chromosome is presented as a cloudy area which, in spite of its haziness, still reveals traces of its double character (Fig. 4). This appearance is difficult to reproduce faithfully in a drawing, but it is very characteristic of late telophasic stages. The haziness gradually diffuses throughout the nucleus, owing to the spreading of the beads or granules (Fig. 5), until there results a faint reticulum with the chromatin almost entirely concentrated in the nucleoli, of which there may be three or more (Fig. 6). This is the so-called 'rest' which probably intervenes between each successive vegetative division.

It may be difficult to discriminate between the phases of nuclei going into, from those coming out of, rest and entering upon prophase. They can, however, usually be distinguished by (1) the gradual blurring of the nuclear contents as the nucleus passes into rest, owing to the fragmentation of the chromatin into finer and finer granules as compared to the sharper definition and generally more active appearance exhibited by nuclei in the onset of prophase; (2) the position of the recently laid down cell-wall between the daughter nuclei. In telophase the nuclei are more or less apposed to the cell-wall, and in a microscopical field of this stage the several pairs of nuclei can as a rule be recognized, whereas in prophase the daughter nuclei move away from the dividing wall towards the centre of the cell.

The earliest indication of the inception of prophase is to be seen in the more active appearance of the nuclei and in the presence of a few large chromatic granules in the reticulum (Fig. 7). The reticulum is very distinct, particularly when stained with Heidenhain (Fig. 8), it having chromatic granules scattered throughout the meshwork, with here and there larger chromatic aggregations. The granules are at first distributed throughout the nucleus, and they then incline to cluster (Fig. 9) and are of varied sizes (Fig. 10). Simultaneously the reticulum gradually passes into a spireme thread.

At this stage the linin threads, containing the beads of chromatin situated at intervals along their lengths, tend to run in parallel pairs; this is the inception of chromosome formation. The parallelisms represent the reassociation of the longitudinal chromosome halves (threads) which had separated during the preceding telophase. As prophase advances, the two

threads associate more and more closely and concentrate, and thus gradually constitute the entire univalent spireme (filament) which eventually segments transversely into the univalent chromosomes. The approaching threads are precisely similar, even to the correspondence of their beading. Later it will be seen that this same phenomenon is exhibited by the threads or associating *halves* of univalent spireme in the presynaptic and synaptic phases (Pl. IX, Figs. 41 and 46) and by the filaments or conjoining entire univalent spiremes in the stages preparatory to second contraction (Pl. X, Fig. 69).

The linin threads become increasingly distinct, and the chromatic beads more definitely arranged, often forming groups of four (Pl. VIII, Figs. 12 and 13). Gradually the chromatin infiltrates the linin threads, causing them to stain chromatically (Figs. 14 and 15). Lengths of threads become closely apposed in pairs (Fig. 16), and the several paired portions (filaments) incline to segregate in a form suggestive of the future individual chromosome segments. In some of these paired segments (filaments) the two sides (threads) may be still beaded and separate for a distance, then becoming closely associated to form a thickened rod—the future chromosome (Figs. 17 and 18). Fine strands still connect the various segments. In the next stage there is an almost complete approximation of the parallel sides (threads) (Fig. 19); the space between them, when visible, is to be distinguished as the line of fission which will separate the daughter chromosomes on the approaching spindle. The spireme filaments, i.e. the *entire* univalent spiremes formed by the association of threads in pairs, are somewhat slight and curve and twist, and the chromatin is evenly diffused throughout their lengths. In some of the nuclei the fission is more evident than in others (Fig. 20).

As the nuclei approach the spindle stage, the filaments become increasingly individualized as chromosomes; one or two nucleoli may still persist. Spindle fibres appear in the cytoplasm and converge towards the nucleus from four points (Fig. 21). These phases are evidently exceedingly quickly passed through, and even in two adjacent nuclei one of them may be in a stage such as shown in Fig. 19, while the other may be in metaphase. With the disappearance of the nuclear membrane, and the invasion of the spindle fibres, the chromosomes concentrate and consequently thicken, and vestiges only of fission can be discerned (Fig. 22). In the process of arranging themselves on the spindle (not figured) the chromosomes are still elongated, but when adjusted they speedily thicken and stain deeply (Fig. 23). The line of fission then opens out and separates each chromosome into its daughter halves, and these recede to their respective poles (Fig. 24). In anaphase (Fig. 1) there is little visible indication of the separation of the halves (threads), preparatory for the next division, inasmuch as fission does not appear until late anaphase or early telophase (Fig. 2). This feature contrasts with the spindle figures of the homotype, where fission

in the daughter chromosomes is very strongly pronounced directly they have separated on the equatorial plate (Pl. XI, Fig. 95). The chromosomes group themselves at the poles (Pl. VIII, Fig. 1), and thus the cycle is completed.

Humphrey (12) describes 'Centrosphären' in connexion with the division figures of *O. regalis*, but they have not been seen in this investigation.

Telophase of the Last Archesporial Division.

In order to interpret the critical and debated phenomena of the heterotype prophases the investigation must be started from the telophase of the preceding archesporial division. It is, however, remarkably difficult to decide whether the archesporial nuclei of any one particular sporangium are in telophase of actually the *last* division before the onset of the heterotype prophase. The growth of the tapetum is not a reliable guide, consequently no correlation can be established between the number of the tapetal layers and the development of the archesporial tissue, or of the subsequently formed spore mother-cells. Matters are further complicated by the rapidity with which the telophase is apparently passed through, because exceedingly few cases can be found which, after critical examination and weighing of all available evidence, can be duly considered to be in this stage. Such sporangia have been exclusively selected for this investigation; the surrounding sporangia having their nuclei, as a rule, in 'rest' or in early heterotype prophase.

Nuclei in late telophase of the last archesporial division are, as would be expected, indistinguishable in character from those of the preceding archesporial mitoses which have already been described (see Text-fig., Nos. 9–12). There is the same splitting of chromosomes into two longitudinal halves (threads), which may be more or less homogeneous or beaded (Figs. 25 and 26). As fragmentation of the chromatin proceeds, the halves (threads) separate widely, and the chromatic contents become distributed throughout the nucleus, and consequently visible chromosome individuality is lost. Fine strands join the chromatic portions together (Fig. 27). The nuclei at this stage are small and have a definite limiting membrane which causes them to stand out sharply from the surrounding cytoplasm. Fig. 28 shows a late stage fixed in strong chromic, which accounts for the chromatin of the now dispersed chromosomes being in relatively more aggregated portions, and staining more deeply—characteristics of this fixative.

From this stage, and onwards to that of the definite heterotype prophase, there is considerable difficulty in unravelling the course of events, as apparently some of the nuclei pass directly from the telophase of the last archesporial division (Pl. VIII, Fig. 29) into the heterotype prophase (Pl. IX, Fig. 35), whilst others pass through an intermediate resting stage (Fig. 34), with the characteristic pre- and post-rest phases (see Text-fig., Nos. 13 and 14). Consequently it is sometimes impossible to decide whether a nucleus is in

telophase or prophase. It might be expected that, as in the earlier archesporial divisions, the position of the recently laid down cell-wall, separating the daughter nuclei, would be a guide to the solution of this problem; but, owing to rapid growth, nuclei undoubtedly in heterotype prophase may be closely apposed to the wall. The following observations have been found to be of some assistance in distinguishing the phases:

(1) The size and shape of the nucleus may be useful, but not infallible, guides. During late telophase and early rest, the nuclei are, as a rule, more or less spherical, and of relatively small dimensions; as prophase proceeds they increase proportionately in volume, and tend to become ovoid in form. (2) A nucleus in telophase has a somewhat definite limiting membrane, but its contour fades away during rest, thus causing the nuclear outline to blend with the surrounding cytoplasm. During prophase the limiting membrane is still indefinite, but the brightly staining contents of the nuclei cause them to stand out in sharp relief. (3) There may be three or more nucleoli in the telophasic stages, but in definite prophase there are seldom more than two. (4) In telophase a clear space, traversed only by isolated threads, is constantly present round each nucleolus, whilst in prophase the nucleoli are more or less entangled in the reticulum.

In the light of these remarks it will be seen that it is not practicable to come to a conclusion with regard to the definition of the stages represented in Pl. VIII, Fig. 30, and Pl. IX, Figs. 31, 32, and 33. The paired beaded threads (Pl. VIII, Fig. 30) are typically telophasic as compared to the pairing of more or less homogeneous threads in prophase. This pairing is very evident in the superficial section (Pl. IX, Fig. 31) taken from the same sporangium as Fig. 30. The three nucleoli and the more or less clear spaces surrounding them are also suggestive of telophase. On the other hand, the large size of the nucleus and its active appearance indicate prophase. A somewhat similar stage is shown in Fig. 32, but in this case little evidence remains of paired beaded threads, though the groupings of the beads in twos or fours and the disposition of the fine linin threads indicate parallel arrangements.

These figures have been intercalated to show the impossibility of identifying certain phases, but, on the other hand, it is feasible to follow out a definite sequence in the passing of telophase into prophase as shown by Figs. 29, 33, and 36. Fig. 29 is a more or less typical late telophase, the chromosome halves (threads) having fragmented into chromatic beads, leaving spaces round the two nucleoli. It is believed that such a nucleus may either proceed directly through an intermediate stage (Fig. 33) into the heterotype prophase (Fig. 36) or may pass through an intermediate rest (Fig. 34). In this particular instance the former is probably its course, for Fig. 33, a nucleus unquestionably in a stage transitional between telophase and prophase, is taken from the same sporangium and even from the same microscopic field as Fig. 29.

The material of this investigation suggests that the resting phase is eliminated more frequently than not, and consequently that the majority of the nuclei pass directly from the telophase of the last archesporial division into the heterotype prophase. This may be accounted for by the fixing of the material having been done solely on warm, growing days at about noon, presumably under conditions of most rapid growth; possibly if they had been fixed under less congenial circumstances there might have been a preponderance of resting phases.

It is proposed to trace the course (1) of those nuclei that pass directly from telophase into prophase, (2) of those that pass through an interkinetal rest before entering upon prophase.

(1) In those nuclei that pass directly from telophase into prophase no complete separation of the chromosome halves (threads) occurs, but traces of paired threads persist. These, therefore, establish the identity between the parallel threads of the telophase of the last archesporial division (derived from the longitudinal splitting of the chromosomes) and the parallel threads of the heterotype prophase, and accordingly testify to the nature of each thread of a pair as representing that of *half a univalent chromosome* (see Text-fig., Nos. 12, 14, and 15).

Fig. 33 is a nucleus in the transition between the telophase of the last archesporial division (Pl. VIII, Fig. 29) and the heterotype prophase (Pl. IX, Fig. 36). Some of the linin threads run in pairs, and some of the chromatic beads are arranged in groups of two and four. Three nucleoli persist. In the next stage (Fig. 36) the clumps of beads have dispersed, leaving a more even reticulum and the general aspect of the nucleus is that of prophase.

(2) Following on the late telophase as shown in Fig. 29, the nucleus may enter the resting stage. Complete rest (Pl. IX, Fig. 34) is characterized by an even distribution of granules and fine threads throughout the nucleus, forming a close, colourless, foam-like reticulum with the chromatic staining contents almost entirely relegated to the nucleoli. The passing of rest into very early prophase (Fig. 35) is recognized by the somewhat more sharply-staining reticulum, by the appearance of chromatic staining granules, and by the generally more active character of the nucleus, thus causing it to be sharply defined from the surrounding cytoplasm as compared to its indistinct contour when in rest. This stage leads on to that of Fig. 36, in which a decided increase in the size of the nucleus has taken place, and the meshes of the reticulum have become extended, showing beads and fine threads joining the beads together.

Heterotype or First Meiotic Division.

In order that the phenomena concerning chromosome formation in the two divisions constituting the meiotic phase may be clearly understood, it

is proposed to give a brief introductory outline of the scheme of events before proceeding with the detailed description (see Text-fig., Nos. 15-35).

It has been shown how, in the archesporial divisions, each pair of parallel threads in the prophase are the reassociating longitudinal halves of a chromosome which had separated during the preceding telophase. The parallel threads concentrate, become closely associated side by side, and thus reorganize a chromosome. They again separate on the spindle and each longitudinal half becomes a daughter chromosome, which will, in its turn, split longitudinally in telophase. Parallel threads, homologous to those of the archesporial prophases, are found in early heterotype prophase, but their complete separation as daughter chromosomes is postponed, and is only finally achieved on the homotype spindle. The paired lengths of threads of the heterotype prophases are balled up in synapsis, and whilst in synapsis the lateral association of the halves (threads) is completed and the entire univalent spireme filament emerges as an apparently continuous thick double thread. This post-synaptic spireme corresponds to the univalent spireme of the somatic prophases before it has segmented transversely into univalent or somatic chromosomes. The univalent spireme of the heterotype division also segments transversely; but before and during second contraction these entire *univalent segments* (filaments) join in pairs to organize a *bivalent* combination, the heterotype chromosome. On the heterotype spindle the whole univalent chromosome of each bivalent pair parts from its fellow, and one passes to either pole. This is therefore a reduction division, for each daughter nucleus will contain half the number of chromosomes typical of the somatic divisions. On the homotype spindle the entire univalent chromosome splits into the two daughter chromosomes. This is the division prepared for by the association in pairs of the half univalent spiremes (threads) met with in the presynaptic and synaptic phases.

Heterotype Prophase to Synapsis.

The earliest definite prophase of the first meiotic division (Fig. 36) shows a more or less coarsely beaded linin reticulum. Some of the fine threads of the reticulum may run in pairs and some of the granules may be arranged in groups of four. These are the first indications of the reassociation of the chromosome halves (threads) which separated during the preceding telophase. From this stage onwards, there is a progressive and decided enlargement of the nucleus, entailing an extension of the clear areas devoid of chromatic contents. Simultaneously the reticulum loses its reticulate character, it becomes drawn out into beaded threads (Fig. 37), and gradually the chromatin of the beads infiltrates the linin, thus causing it to stain evenly and to assume more definitely the spireme character (Fig. 38). This spireme thread shows a gradual but conspicuous parallel arrangement of portions of its lengths (Fig. 39). The pairing becomes more accentuated

(Fig. 40) until, as in Fig. 41, long lengths may run parallel to one another, and these may be connected by paired beads at intervals At this stage the withdrawal of the nuclear contents preparatory to synapsis is first indicated; in Fig. 41 the spireme (thread) has retreated from a small area of the periphery in the NW. region and in other parts has become slightly drawn together. As the spireme (thread) withdraws from the nuclear periphery it becomes increasingly thrown into loops, suggesting a continuous thread (Fig. 42); but it is not possible to prove this supposition. At this stage the spireme (thread) resembles a loosely tangled skein of wool with threads running very closely parallel (Fig. 44), sometimes actually in contact (Fig. 42), whilst others twist and loop over one another (Figs. 42 and 43).

When the nucleus has passed into the early synaptic stage (Fig. 45) the knot, in favourable preparations, is seen to consist of fine threads, the cut ends of which appear as small beads of chromatin. The association of the sides of the loops (i. e. of two threads) can be recognized towards the thinner margins of the knot; the associations are obscured towards the centre by the close tangle of threads. The similarity of the approximating sides (threads) is striking (Fig. 46); so alike are they that they would be regarded as splitting instead of associating had not their origin been traced. They also appear exceedingly taut and strained. Both these features will again be found to be characteristic of the univalent segments (filaments) of spireme when they conjoin in pairs.

Synapsis.

The synaptic stage of *Osmunda* is, as a rule, far more instructive than in the majority of plants, where it often happens that its densely staining intricate mass makes its structure impossible to resolve. Such is also the case in *Osmunda* when fixed with strong chromic, but with strong Flemming and Hermann the individual threads can be clearly seen. Merkel fixative has a variable effect; sometimes it yields a clear, and sometimes a dense, appearance during synapsis.

During synapsis, the association of threads in pairs, prepared for during the presynaptic stages, is consummated. The complete sorting out and association in pairs, side by side, of the threads of the whole skein of half univalent spireme (thread) is achieved, and hence the spireme filament which issues from synapsis is, throughout its length, of an *entire* univalent nature. If an early synapsis before association (Fig. 45) be compared with a late synapsis after association (Fig. 49), when the spireme (filament) is beginning to unravel and to invade the nuclear cavity, it will be observed that the difference in thickness of the spiremes is most striking, and that there is no difficulty in distinguishing the two phases. The spireme thread, therefore, in the early synaptic stages, before association, is not only half the thickness, but is also of necessity double the length of the spireme filament of the later synaptic stages when association is accomplished.

It is only very rarely that association of a long length of spireme thread is to be seen proceeding in a nucleus, but such cases, when present, are most striking. The half univalent spireme (thread) is thrown into loops which are orientated towards one portion of the nuclear periphery, and there the sides of the loops (i.e. threads) are seen to be associating in pairs (Figs. 47 and 48). This investigation has necessitated the examination of a very large amount of material, and only these two diagrammatic examples have been found. If the spireme loops are orientated solely towards the nuclear poles, then the chance of cutting the nucleus at the precise angle to demonstrate the phenomenon would be remote. In the majority of nuclei at this stage, association can be detected as taking place between lengths of spireme thread in the synaptic knot, but these cases are naturally not nearly so convincing as in the nuclei figured. Grégoire (10) has published a beautiful drawing (Planche I, Fig. 24) of this polarization showing the association in a most graphic manner. He writes: 'C'est surtout dans l'*Osmunda regalis* que nous avons rencontré les plus beaux cas de dualités d'accolement. . . . Dans la Fig. 24 on voit des filaments [1] minces nettement orientés vers un pôle du noyau, chose assez rare dans les sporocytes—nos figures en apportent le premier cas aussi clair, à notre connaissance' (p. 378). He interprets the association of threads in synapsis as the association of two lengths of *entire* univalent spiremes, each associating thread representing an *entire* somatic chromosome; whereas the conclusion arrived at during the present investigation is that each length of associating spireme represents the longitudinal *half* of a univalent spireme or univalent chromosome.

The association of threads in pairs to form the spireme filament is completed before the spireme begins to come out of synapsis; the emerging spireme filament, therefore, is *double throughout its length*—no single unassociated threads remain (Fig. 49). Accordingly from late synapsis onwards the nature of the spireme is that of an *entire* univalent filament, i. e. the product of the lateral association of the two longitudinal halves (threads) of univalent spireme, but separation between the halves (threads) may still remain more or less evident. This spireme filament is homologous with the continuous spireme filament of a somatic prophase, before it segments transversely into the somatic chromosomes.

The nucleolus occasionally buds during synapsis. No typical formation of chromatic bodies has been observed, but von Derschau (2) has figured and described them in *O. regalis*.

Early Hollow Spireme Stage.

As the univalent spireme filament comes out of synapsis (Fig. 49) fission is, as a rule, conspicuous. Sometimes the separation of the two associated threads is considerable, sometimes it is shown merely by the

[1] 'Threads', according to the nomenclature adopted in this paper (see foot-note, p. 159).

shading in the beading, whilst at still other times the sides (threads) are so closely appressed as to form a single row of beads. Fig. 50 shows a Hermann fixed superficial section of the loosening of synapsis. In this nucleus every portion of the spireme filament exhibits fission (i. e. separation) and that of a very beaded kind characteristic of Hermann preparations, whilst in strong Flemming preparations the sides (threads) are more homogeneous (Fig. 51). The spireme filament distributes itself throughout the nucleus (Fig. 51), the so-called 'hollow spireme' stage, and as it invades the nuclear cavity it becomes thrown into loops (Fig. 52), and these are often orientated round the nucleolus (Fig. 53). It is exceedingly difficult to decide whether free ends are present, but the evidence points to an affirmative, and at a slightly later stage they are certainly to be found. From this stage onwards the spireme filament becomes more uniformly homogeneous, and fission rapidly closes. Figs. 53 and 54 have been taken from sporangia in which the nuclei of half of the sporangium show fission, whilst in those of the other half fission has already closed (Pl. X, Fig. 59).

Perhaps it may not be out of place here to make a slight digression and allude to the widely separated stages in the progress of nuclear division that may be found in a single sporangium, testifying to the rapidity with which many of these phases are passed through. In a single sporangium some nuclei may be in the pre-second contraction phase, whilst others are in diakinesis (Pl. XII, Fig. 101); or half of the nuclei may be in second contraction and the other half in metaphase. These rapid transitions increase the difficulty in interpreting the complicated stages leading to second contraction. Wilson Smith (18) has given some interesting data on this subject, and it is evident that in his plants growth was not so rapid as in the material collected for the present research. His material of *O. regalis* was fixed in northern Indiana, and he calculates that the resting and early spireme stages last for about two weeks, synapsis for three or four days, whilst the two meiotic divisions are effected in quick succession, within a period of two or three days.

Later Hollow Spireme to Second Contraction.

To describe clearly the series of nuclear phases between hollow spireme and second contraction is a difficult task, for not only do the spireme filaments show great diversity according to the fixative used (with strong chromic the swelling is considerable), but even adjacent nuclei in a sporangium reveal strikingly different characters.

The principle to be borne in mind is, that as the nuclei pass into second contraction *an arrangement for the conjunction in pairs of entire univalent spireme segments* (i.e. of filaments) *is evolved.* This conjunction may be in all stages of completion before second contraction supervenes, but it is finally consummated in the massing of second contraction, during

which the course of events becomes obscured. Whereas during synapsis the association of the *halves* of univalent chromosomes is completed, so during second contraction the conjunction in pairs of *entire* univalent chromosomes is achieved.

It is not possible to find a nucleus which shows diagrammatically the conjunction of all its spireme filaments, but by working through every nucleus of a sporangium at this stage, isolated instances may be observed. In a single nucleus several pairs of filaments may show conjunction, or perhaps only one pair may be conjoining, or there may be no trace of conjunction whatsoever. It is only by comparing numbers of nuclei, and by checking them with those of pre- and post-stages, that it is possible to arrive at a conclusion on the matter.

When the nucleus is in the late hollow spireme stage, the first indication of conjunction may be observed in well-fixed preparations. These early stages, so unmistakable in the particular material on which this investigation is based, had never been observed in the fixations of former years. Moreover, the phases subsequent to second contraction, which seemed hitherto to defy interpretation, are here intelligible and clear, and there are many graduated transitional phases to be seen. The advantages which have made the elucidation of these difficult and crucial stages possible are due to the successful fixation carried out in the manner already described (p. 136).

The first sign of conjunction is recognized by the tendency of the sides of the loops (i. e. of spireme filaments) to incline towards one another, and at the points where they are bent in closely together they become joined by very fine connecting strands (Pl. IX, Figs. 54 and 55). The surface of the spireme is slightly drawn out at the place of attachment of these strands. This is the first step in the combination of entire univalent spiremes (filaments) or chromosomes in pairs to form the bivalent spiremes which will become the heterotype chromosomes. In Fig. 55 the sides of the loops are connected at one point suggestive of a future U-shaped chromosome; Fig. 56 typifies the future X-shaped chromosome; whilst Figs. 57 and 58 show a looping over of the spireme filament characteristic of other heterotype chromosome figures.

These studies of early conjunction of univalents (filaments) show degrees of fission (i.e. traces of the two original separate threads of which the univalent spireme is composed) in each univalent spireme, but from this stage onwards to the coming out of second contraction fission is usually closed. *Fission is never visible in the substance of the filaments when they are closely conjoined* (Pl. X, Figs. 60 and 63), though it may be evident in the filaments which are still independent (Figs. 65 and 66).

Fig. 59 is taken from the same sporangium as Fig. 53, but is in a decidedly later stage. In this univalent spireme (Fig. 59) complete

coalescence of the approximated threads has occurred, and there is considerable thickening of the spireme filament. The nucleus has been cut somewhat superficially, but the preparation for conjunction is manifested both in the looping over and drawing together of the sides of the loops, and in the fine connecting transverse strands.

The stage when the univalent spireme (filament) is uniformly spread throughout the nuclear cavity appears to be of short duration, for almost immediately it tends to aggregate preparatory for second contraction (Fig. 60), and at the same time becomes stretched and smoother in outline. It now shows various degrees in the process of conjunction in pairs; some lengths may be merely running parallel, whilst others, though still separate, are joined at intervals by fine strands (Fig. 59); others form a closed ring (Fig. 61); others are twisted over one another (Fig. 61); whilst others are closely conjoined side by side for some distance (Fig. 60) and then diverge. If each nucleus in a sporangium at this stage be examined, it will be found that the spiremes in the majority show some signs of conjunction. Fission is still occasionally visible as in the loop of Fig. 62. Moreover, there may be a straightening out of the loops and also beautiful anastomosis, but as these figures are somewhat aberrant they have not been published.

The massing of the spireme filaments preparatory for second contraction increases (Fig. 62), and at the same time they withdraw from the periphery of the nucleus and definitely become transversely segmented (Figs. 62 and 63). Conjunction meanwhile proceeds (Fig. 63). Nuclei cut superficially show segments of conjoining spireme filaments (Pl. X, Figs. 64, 65, and 66) which bear a striking resemblance to the future completely organized heterotype chromosomes (Pl. XI, Figs. 80 and 81) except for their slightness as compared to the concentrated mature segments.

It may here be emphasized that the breadth of the spireme filament affords no criterion whatsoever on which to build any calculation as to the stage reached, except in the extreme case mentioned above, and also in the pre- and post-synaptic spiremes before and after association. Thin spireme is so often due solely to tension, as in associating half univalent spiremes (threads) and conjoining univalent segments (filaments), and directly the strain is relaxed the spiremes shorten and thicken.

Fission is apparent in the free portions of univalent sides (filaments) of several of these chromosome-like combinations (Figs. 64, 65, and 66). Conjunction of the univalents (filaments) becomes increasingly close. Figs. 67, 68, and 69 have all been taken from the same sporangium, and Figs. 67 and 69 from the same microscopic field. In Fig. 67 comparatively little conjunction has taken place, whilst in Figs. 68 and 69 conjunction is advanced. Lawson (14) has published two identical stages in *Smilacina* (Pl. I, Figs. 15 and 16). He states that the large number of threads[1] present in his Fig. 15

[1] 'Filaments', according to the nomenclature adopted in this paper.

makes it quite clear that 'the reduction in the number of chromosomes has not yet taken place', whilst in his Fig. 16 the spireme threads[1] 'are segregating in pairs preparatory to a lateral union' (p. 625).

When the majority of the spireme filaments have conjoined in pairs (Figs. 68 and 69), they necessarily occupy a more restricted area than when they were independent of one another (cf. Figs. 60, 62, 63, and 67 with Figs. 68 and 69). The nucleus at the same time decreases considerably in size. The conjoining spireme filaments stain uniformly and have a taut and strained appearance prior to close conjunction (cf. Figs. 59, 63, and 67 with Fig. 69). Close conjunction bears a striking resemblance to fission, the two approaching univalent spiremes (filaments) being identical even to their beading (Fig. 69). It is only by following every stage, and by constant comparison, that these pre-second contraction phases can be interpreted and the fact substantiated that one is here dealing with *conjunction* and not with *separation*. It will be remembered that the associating halves of univalent spireme (threads) in presynaptic prophases exhibit precisely similar characters.

The conjoining stages appear to have been missed by many cytologists, who have therefore concluded that these paired filaments going into second contraction are *dis*-joining and not *con*-joining, and hence that this separation is the opening out of the fission seen in the post-synaptic spireme. Grégoire (10) and Yamanouchi (19) have both come to this conclusion with regard to *Osmunda*. Farmer and Moore (8), on the other hand, described the coming together of the sides of the loops in the stages leading to second contraction, and they note that they approximate closely 'and thus simulate an appearance of a longitudinal fission' (p. 520). It is proposed to refer to this point again in the 'General Considerations'.

Gradually (Figs. 70 and 71) the greater part of the spireme filaments become collected together and lose their visible identity in the massing of second contraction, but isolated portions may remain independent and free (Fig. 71). In this nucleus (Fig. 71) the two outlying spireme filaments show a twisted conjunction, but in Fig. 72 some of the filaments, not involved in the aggregation of contents, are widely separated, whilst others are closely conjoined. It is suggested that close conjunction of two filaments (i. e. of two univalent spiremes) may, in some instances, never take place at all, for it is not unusual to find bivalent chromosome-like entities lying outside the mass of second contraction; these apparently concentrate and thicken, and thus take a short cut to becoming heterotype chromosomes.

The main and final process of conjunction, involving the sorting out and conjoining in pairs of *all* the univalent segments (filaments), takes place during second contraction, whereas the association of the pair of threads which together make up the univalent spireme is consummated during synapsis.

[1] 'Filaments', according to the nomenclature adopted in this paper.

Coming out of Second Contraction and Diakinesis.

From the stage of second contraction, and onwards up to the close of the homotype division, the nuclear phases are comparatively straightforward, are simple of interpretation, and afford little ground for debate.

There is no difficulty in distinguishing between the nuclei issuing from second contraction, and those about to enter that phase; for in working through the post-second contraction events, bivalent chromosomes in varying stages of evolution will be seen. Further, the univalent chromosome segments often issue from second contraction in detached portions, and these speedily join end to end (Fig. 73). Moreover, the spore mother-cells now begin to separate. It has been found in these preparations, in which the method of fixation has largely eliminated artificial contraction, that the rounding-off of the spore mother-cells occurs at a considerably later phase than would appear to be the case when studying material which had not been so well fixed.

As second contraction loosens, the bivalent segments begin to sort themselves out (Fig. 73). Some of the univalents (filaments) are still closely conjoined, whilst others are more or less separated, and it may be possible in isolated instances to distinguish the separate bivalent combinations, i. e. the future heterotype chromosomes. Some second contraction figures loosen directly into more or less organized thick, concentrated chromosomes (Fig. 74), whilst in others the univalents are more slender, and are in process of disjoining (Fig. 75).

As the univalent chromosomes of each bivalent combination separate, they thicken (Fig. 76) and stain as more or less homogeneous bodies. The nuclear surface then increases considerably and the chromosome segments are dispersed at its periphery. Some of the bivalent chromosomes are clearly individualized, whilst others are not completely organized. Fission can only rarely be detected at this stage (Fig. 77). This is the reappearance of the fission which was evident in the univalent post-synaptic spireme, parting the threads which had associated during synapsis, and it will eventually result in the separation of the daughter univalent chromosomes on the homotype spindle.

The chromosomes concentrate rapidly (Fig. 78); the small granules and globules which are often seen scattered in the nucleus and even attached to the chromosomes are probably fragments of dissolving nucleolus.

In fortunate preparations spindle fibres are seen to radiate towards the nucleus from four poles which appear as cones in the cytoplasm (Fig. 79), as in *Polypodium vulgare* (7) and other ferns. Strasburger (17) has figured a multipolar spindle origin (Pl. IV, Fig. 178). Wilson Smith (18) occa-

sionally found tripolar spindles, but he considers them to be abnormal stages in spindle development.

The chromosomes retreat before the encroaching fibres towards the centre of the nucleus, and fission is sometimes evident in the univalent segments (Pl. XI, Fig. 80). The nuclear limiting membrane disappears, the fibres invade the nucleus (Fig. 81), and the chromosomes collect towards the centre of the nucleus.

Heterotype Metaphase, Anaphase, and Telophase.

The chromosomes then arrange themselves on the spindle (Fig. 82), which has now become bipolar. The number of chromosomes as seen in polar view of an equatorial plate of *O. palustris*, var. *aurea*, is apparently 20 (Fig. 83); the reduced number both in *O. regalis* (17, 11, 13) and in *O. cinnamomea* (19, 13) is 22.

Disjunction of the entire univalent chromosomes of each bivalent pair takes place on the equatorial plate, and it is immediately succeeded by a cleavage in the substance of each entire chromosome, widely separating it into diverging halves. This is the first sign of dissociation of the threads of spireme which associated in pairs during the presynaptic and synaptic stages, and is therefore the opening out of the fission so clearly seen in the entire univalent post-synaptic spireme (filament).

Fission in the univalent chromosomes as they proceed to the heterotype spindle poles is so extensive as to split each chromosome into a V, the apex of the V pointing to the pole (Fig. 84) (see Text-fig., No. 25). This precocious cleavage may be attributable to the fact that *entire* univalent chromosomes are passing to the poles, a phenomenon peculiar to the heterotype mitosis. These daughter chromosomes will finally separate on the homotype spindle. The cleavage continues to be very marked when the chromosomes are looked at in polar view of early anaphase (Fig. 85), and it causes each chromosome to be double, thus making it difficult to obtain an accurate count.

The chromosomes draw together and a further and new fission appears in the substance of each daughter chromosome, giving it a fenestrated or vacuolated appearance (Fig. 86) (see Text-fig., No. 26). This new fission in the daughter chromosomes of the heterotype telophase is homologous with the fission in the daughter chromosomes, of a somatic telophase. It is prophetic of the *post-homotypic* division, and reappears in the early anaphase and telophases of the homotype mitosis, thereby splitting the daughter chromosomes (Fig. 98) into longitudinal halves (threads) (see Text-fig., Nos. 31, 33, 34, and 35).

This double fission becomes intelligible when it is remembered that *entire* univalent premeiotic chromosomes are distributed to the poles of the heterotype spindle, instead of *half* or *daughter* univalent chromosomes, as in somatic divisions. Consequently, not only does the whole univalent

chromosome split into its daughter halves, but the daughter halves themselves conform to their normal procedure in somatic telophases, and split into longitudinal halves (threads). This fact once more illustrates the dual nature of each chromosome.

The splitting of the daughter chromosome halves initiated by the fenestration proceeds (Fig. 87) and the identity of the chromosomes is lost to view in paired beads and parallel threads (Fig. 88). Simultaneously a nuclear limiting membrane makes its appearance.

In tracing the series of stages between the heterotype telophase and the onset of the homotype prophase, the presence or absence of the nuclear limiting membrane and the evolution of the cell-plate separating the daughter nuclei are found to be useful guides in the reconstruction of the sequences. The phases between the two divisions are apparently quickly passed through, for it is not uncommon to find heterotype anaphases interspersed with homotype metaphases in the same sporangium.

Following the dissolution of the chromosomes comes their reconstruction, and this may be regarded as the beginning of the homotype division.

Homotype or Second Meiotic Division.

The homotype may justly be regarded as a continuation of the last *premeiotic division*, and consequently the heterotype represents an interpolated phase in which the numerical reduction of the chromosomes is accomplished.

The univalent spireme filament of the homotypic prophases is homologous with the univalent spireme filament of the heterotypic prophases, and this in turn is derived from the reassociation of the longitudinal halves of univalent chromosomes (threads) that had split apart during the preceding telophase of the last premeiotic division. These halves (or threads) do *not* dissociate during the heterotype division, but their separation takes place on the homotype spindle (see Text-fig., No. 30).

The points of difference between a homotypic and a somatic division are: (1) the homotype possesses half the somatic number of chromosomes; (2) the homotype is endowed with *entire* univalent chromosomes from the preceding heterotype spindle, instead of with *half* univalent or daughter chromosomes; (3) the homotype prophases show a very precocious splitting of the univalent spireme as compared to somatic prophases, or, in other words, the daughter chromosomes, which will separate on the homotypic spindle, are dissociated at a very early stage.

The onset of the homotype prophase is recognized by the chromatic contents becoming concentrated into rounded masses in excess of the x number of chromosomes (Fig. 89). This is not remarkable, as double the reduced number of chromosomes split into longitudinal halves (threads) during the preceding heterotype telophase owing to the splitting of each entire univalent chromosome into daughter chromosomes (Figs. 84 and 85),

followed by a further longitudinal splitting of each daughter chromosome into halves (threads) (Figs. 86 and 87). Consequently, as the longitudinal halves (threads) severally associate in pairs, they bring about the reorganization of the double number of chromosome segments (see Text-fig., Nos. 25–28).

It has not been possible to find stages in the building up of the chromatic masses, so quickly do the longitudinal halves (threads) appear to reassociate upon their partial separation, but it is assumed that each of these masses is a filament, derived from the reassociation of the split halves (threads) of a daughter chromosome of the heterotype telophase. This view is confirmed by the fact that throughout the homotype prophases, the daughter univalent chromosomes, instead of being closely associated as in somatic prophases, are organized as more or less independent individuals, and they even take up their position as such on the equatorial plate.

These chromatic masses are arranged round that portion of the periphery of the nucleus which is remote from the cell-plate, leaving a central clear space. Accordingly, they emphasize that particular area of the nuclear limiting membrane, whereas the boundary of the nucleus towards the cell-plate is undefined and gives access to cytoplasmic strands. Wilson Smith (18) also remarks on the bunching together of the chromatin on the side of the cell remote from the greater mass of 'kinoplasm'.

The nuclear membrane gradually disappears, the chromatic contents collect closely together (Fig. 90), and the further events are involved in some obscurity. Spindle fibres radiate into this mass, and the chromatic segments become more and more indeterminate (Fig. 91). The segments loosen out along the fibres, and the nucleus elongates at right angles to the cell-plate of the heterotype division (Fig. 92). The evolution of the spindle fibres proceeds (Fig. 93), and the chromosome-like segments are greatly elongated. They become almost completely divided by fission into longitudinal halves (Fig. 93); in fact, the daughter chromosomes take up their position as independent individuals on the equatorial plate (Fig. 94) instead of dividing in the ordinary manner only when arranged on the spindle.

As the daughter chromosomes move away from one another and proceed towards the spindle poles (Fig. 95) fission once more immediately appears in them, often resulting in a separation of each of them into complete halves (threads). This is the fission prepared for by the alveolization of the daughter chromosomes in the heterotype anaphase. Arrived at the poles (Fig. 96) the tension slackens, and the chromosomes draw together in the figure of a rosette, as seen from a polar view. They thicken considerably and for a time appear homogeneous, fission being temporarily obliterated. As anaphase passes into telophase (Fig. 97) the chromosomes separate from one another and fission once more reappears; each chromosome divides into two halves (threads) which are at first homogeneous, but rapidly become beaded (Fig. 98). In Fig. 97 one chromosome, in advance of the

others, has already split into two parallel rows of beads. A nuclear limiting membrane makes its appearance (Fig. 99), the skeletons of the quondam chromosomes become less and less recognizable as their beaded sides separate, and thus inaugurate a reticulum (Fig. 99). The beads gradually become distributed throughout the nucleus (Fig. 100), which thus passes into the fine reticulate, or resting, stage found in the cells of the spore tetrad.

The predominating feature of the homotype division lies in the precocious separation of the daughter chromosomes, suggesting weak cohesion between them. Possibly some physical conditions associated with the reception into the nucleus of entire premeiotic chromosomes during the previous telophase of the heterotype division may be responsible for this phenomenon.

Synangia.

Five cases of synangia have been found, three in *O. palustris*, var. *aurea*, and two in *O. palustris*, var. *undulata*. Of these one is in archesporial division (Pl. XII, Fig. 102) the others in the first meiotic division (Figs. 103, 104, 105, 106). One side of the synangium may have its spore mother-nuclei in a considerably more advanced stage than the others (Figs. 105 and 106).

It is interesting to note that Bower (1) has described synangia as occurring not unfrequently in *O. regalis* (Pl. III, Fig. 51); he has also found them in *Gleichenia* (Pl. I, Fig. 31), and mentions that they may be observed occasionally in *Todea barbara*.

General Considerations.

No attempt will be made to touch even the fringe of the vast literature which has grown up round the hotly debated question concerning the mode of origin of heterotype chromosomes. It is merely proposed (1) briefly to recall the main differences in the views held by telosynaptists and parasynaptists, (2) to suggest a possible explanation for these different views by showing how variously the details of mitosis may be modified, (3) to consider in particular the evidence afforded by the mitoses of *Osmunda*.

1. *The Main Differences between the Telosynaptic and Parasynaptic Views.*

Farmer (6) has drawn attention to the confusion which has arisen round the terms 'telosynapsis' and 'parasynapsis', and sums up the position in a few cogent sentences. He shows that by emphasizing a point of comparative unimportance, these terms 'have led to a misconception on the part of many people, as to the really fundamental differences which still divide the two schools of investigators'. There is no essential difference between a lateral approximation achieved by the twisting together of the sides of a loop, 'and an approximation produced by the coming together,

in pairs of chromosomes hitherto disunited, nor is it a matter of any importance whether the approximation occurs at a somewhat earlier or later period in mitosis. The really vital question at issue between the two schools does not, as a matter of fact, consist in Telosynapsis *v.* Parasynapsis as etymologically understood, *but upon the interpretation to be placed on the much earlier stages of prophase* in the heterotype mitosis.'

Telosynaptists and parasynaptists [1] are agreed as to the evolution of the somatic chromosomes.[2] They acknowledge the splitting into longitudinal halves (threads) of each daughter somatic chromosome during telophase; the separation of the halves (threads) during interkinesis, their reassociation during the ensuing prophase, and their final separation as two daughter chromosomes during metaphase, each preparing to split again during telophase and thus completing the cycle.

Those who advocate the *telosynaptic* theory for the origin of the *heterotype* chromosomes regard the parallel threads of the heterotype prophase as homologous with those of the somatic prophases, namely that each parallel thread of a pair represents *half* a somatic chromosome which separated from the other half in the preceding telophase. On the other hand, parasynaptists regard the parallel threads of the heterotype prophase as the pairing of *entire* somatic chromosomes. This is the fundamental and vital difference in the two views, and governs the interpretation of subsequent stages. Telosynaptists accordingly regard the spireme that issues from synapsis as *univalent*, and claim a subsequent secondary conjunction of paired portions of univalent spireme, during the second contraction stages, to form the typical bivalent or heterotype chromosome. Parasynaptists, on the other hand, regard the spireme that issues from synapsis as already *bivalent*, and consequently believe that this spireme itself splits to form the typical univalent segments of the heterotype chromosome. Lastly, as a final corollary, telosynaptists hold that the paired threads, which associated during synapsis, separate on the *homotype* spindle, whereas parasynaptists maintain that they separate on the *heterotype* spindle.

2. *Modifications in the Details of Mitosis.*

It is evident that the root of the difficulty lies in the fact that as yet no single type has been found, whether animal or plant, which shows clearly and straightforwardly, with no elimination or disguising of phases, the whole series of events passed through during the heterotype mitosis. Each form has its distinctive cytological characters, and its nuclei may show one particular phase with exceeding clearness, whilst the pre- or post-stages

[1] These terms are employed here because they have become so widely used, and not because they are regarded as correct or even appropriate.

[2] It has been noted (p. 138) that a modification of the characteristic type of somatic division may be found (as in *Primula*) in which fission tends to remain closed until it splits the chromosomes apart on the equatorial plate.

may be confused. A well-defined phase, taken without its context, may be misleading and give rise to an error of judgement. For example, the parallel threads of the heterotype prophases may be exceedingly striking and definite, but it is impossible to offer a fully reasoned statement as to their homology, if an interkinetal rest exists between the last premeiotic division and the heterotype prophase during which all visible continuity is lost. Or again, fission in the post-synaptic spireme may be very evident, but this stage may be succeeded by most confused hollow spireme and second contraction phases, thus obscuring the conjunction of univalents. This has led to the very commonly expressed inference that the disjunction of univalents after second contraction is the opening out of the fission observed in the substance of the post-synaptic spireme.

It is believed that no conclusion can be drawn from the study of a series of nuclear phases of any one particular animal or plant. In order to expand this suggestion, it is proposed to compare the critical stages of the heterotype division in four plants, *Galtonia* (3), *Primula* (4), *Crepis* (5), and *Osmunda*. Each exhibits not only individually distinctive characters which dominate its mitoses, but shows considerable variation in the details.

The most instructive point in *Galtonia* is the direct passing of the telophase of the last premeiotic division into the meiotic prophase with no intermediate rest; consequently the origin of the parallel threads of the heterotype prophases can be traced directly to the longitudinally split chromosomes of the preceding telophase. This fact substantiates the nature of each thread of the parallel pair as representing the longitudinal *half* of a somatic chromosome. None of the other three plants show this all-important character so clearly. In *Osmunda* the homology can occasionally be traced, but not in so indubitable a manner, and a rest may intervene between the two divisions. In *Crepis* and *Primula* there is invariably a complete rest during which all visible chromosome continuity is lost.

To proceed to synapsis. This stage is only really clear in *Osmunda*, on account of the distinct individuality maintained as between the threads. Under favourable conditions, many of the parallel threads of presynapsis can be seen, in a single nucleus, to be simultaneously in the act of closely associating in pairs. The contrast between the fine associating threads going into synapsis and the resultant thick emerging spireme filament is exceedingly striking. In *Galtonia*, *Primula*, and *Crepis*, the density of the synaptic knot obscures the course of events there taking place.

To pass on to the complex stages between hollow spireme and early diakinesis. *Primula* shows the conjunction of filaments in pairs, i.e. of portions of univalent spireme, most clearly and diagrammatically. The figures are exceedingly sharply defined, and the univalent spireme filament is homogeneous, showing no fission, which simplifies the following out of the process. Moreover, in two species, *P. floribunda* and *P. kewensis*

(seedling), second contraction is omitted, so that the sequence of phases extending from the first indication of the conjunction of univalents (filaments) to the realization of the mature bivalent chromosomes can be traced without intermittence. In *Osmunda* the conjunction of filaments in late hollow spireme is conspicuous in well-fixed preparations, but the fact that fission may persist nearly to second contraction adds a perplexing feature. In *Galtonia* there is no convincing evidence for conjunction, but as the nucleus approaches second contraction the univalents (filaments) tend to conjoin in pairs to form bivalent segments. In *Crepis virens*, notwithstanding that the haploid number of chromosomes is but 3, it is impossible to trace their evolution, owing to the viscous character of the nuclear contents, and also to the fact that the hollow spireme stage is usually omitted, the nucleus passing almost directly from synapsis into second contraction.

From the foregoing remarks it will be realized that no one of these four plants is completely satisfactory throughout its phases, but if the telophases of the last premeiotic division and the early heterotype prophases of *Galtonia* could be combined with the later presynaptic and synaptic phases of *Osmunda*, and the hollow spireme to diakinesis stages of *Primula*, a completely coherent and intelligible series of nuclear phases would be established.

3. *The Evidence afforded by* OSMUNDA.

The conclusions of several investigators with regard to the origin of the heterotype chromosomes in *Osmunda* are somewhat at variance.

In 1900 Strasburger (17) incorporated a short account of the heterotype division of *O. regalis* in a paper dealing with a variety of subjects. He does not inquire into the manner in which the chromosomes are reduced, as he assumes the heterotype prophase to be predestined to evolve the x number. The fission which appears in the spireme, soon after it has emerged from synapsis, he regards as the premature splitting apart of the two univalent chromosomes which will separate on the equatorial plate of the heterotype spindle. Great emphasis is laid on the fact that this separation is immediately followed by a second longitudinal fission for the homotype division.

Farmer and Moore (8) in 1905 showed that a similarity in the process of reduction occurs in animals and plants, and *O. regalis* was one of the plants selected. They describe the close conjoining of the sides of the loops at the onset of second contraction, each side eventually forming a univalent segment of the heterotype or bivalent chromosome. Further, that 'the homotype mitosis is associated with the completion of the longitudinal division of the chromosomes already incepted during the prophase of the heterotype division' (p. 505).

In 1907 Grégoire (10) cited *Osmunda* as strongly supporting the parasynaptic theory, and in this paper appears his historical figure of a nucleus

in synapsis showing the 'filaments minces'[1] orientated towards one pole of the nucleus and associating in pairs. He regards this stage as the pairing of entire univalent chromosomes. With Strasburger, he considers the fission in the post-synaptic spireme as the separation of these same entire univalent chromosomes which had come together during synapsis and which will separate at the heterotype metaphase.

In 1910 Yamanouchi (19) published a paper on *O. cinnamomea*, and his views coincide with those of Grégoire. He traces the duality of the threads,[2] from early prophase to their separation as daughter chromosomes on the heterotype spindle. He shows that 'the threads come out of the network as two independent threads from the start' (p. 5); that they come to lie parallel to one another, and that these 'double threads' shorten and thicken, and a pair of such shortened and thickened 'threads' become a bivalent chromosome (p. 6).

It is evident that neither Strasburger, Grégoire, nor Yamanouchi noticed the conjunction of segments of entire univalent spireme (i.e. of filaments) in the post-synaptic stages, and consequently were unaware of the complications thus introduced, and Grégoire and Yamanouchi consequently assumed that the striking pairing of threads in presynapsis and synapsis was the pairing of entire univalent chromosomes.

After years of hesitation the writer has come to the firm conclusion that *Osmunda* affords a striking piece of evidence in favour of the 'telosynaptic'[3] theory of the origin of the heterotype chromosomes. It is possible to identify all the important stages illustrated by the previously mentioned workers, namely the parallel filaments (of Grégoire) in the presynaptic prophases, the association of these filaments in pairs during synapsis resulting in the emerging thick spireme, and the separation of the univalent segments as they come out of second contraction to organize the typical heterotype chromosomes. If this really represents the complete sequence of events, and if the fission in the post-synaptic spireme is that which eventually parts the univalent chromosomes on the heterotype spindle, how has the numerical reduction of the chromosomes come about? The only possible answer is that the paired filaments (of Grégoire) of the

[1] Grégoire (1910, Les Cinèses de maturation dans les deux règnes. La Cellule, xxvi) calls the 'threads' (term used in the sense adopted in this paper) of heterotype prophases 'filaments minces', and considers each 'filament mince' to be an *entire* somatic chromosome. He thus describes synapsis: 'C'est le stade de *noyau leptotène* (Winiwarter, 1900) ou *leptonema* (Grégoire, 1907), ou noyau à filaments minces . . . l'association deux à deux des filaments minces des noyaux leptotènes et un rapprochement graduel des deux filaments associés, donnant origine aux anses épaisses des noyaux pachytènes. . . . Nous avons (1907) proposé, pour ce stade, le nom de noyaux *zygotènes*' (= noyaux *amphitènes* of Janssens) (p. 237).

[2] Yamanouchi (19) retains the term 'threads' throughout the heterotype division. He considers the pairing of 'threads' in synapsis to be the coming together of two *entire* homologous somatic chromosomes which separate on the heterotype spindle.

[3] See foot-note on p. 156.

presynaptic prophases must represent the pairing of entire somatic chromosomes. This brings one to the bed-rock of the problem, and it will be discussed in detail.

From evidence supplied by both schools of cytologists, it is clear that, as a general rule,[1] the somatic chromosomes in telophase apparently disorganize by first dividing into longitudinal halves (threads), which gradually become dissipated throughout the nucleus. Prophase, therefore, entails a reassociation of these halves for the reorganization of each individual chromosome. If this dual nature of the chromosomes be borne in mind, it will be realized that in *all* prophases, whether somatic or meiotic, there must be a coming together of the halves (threads) dispersed during the preceding telophase involving a pairing of threads. Consequently, that parallel threads should be present in all nuclei, including those of parthenogenetic eggs (15) and gametophytes (9), is only a normal event, and it bears no relation whatsoever to the pairing of *entire* homologous chromosomes.

Grégoire (10) and his followers promote this view with regard to somatic prophases, but, on the other hand, they regard the paired threads of the heterotype prophases as the pairing of filaments representing *entire* homologous chromosomes. Such a theory postulates the complete rearrangement of the elements of the last premeiotic telophase, for which, however, there appears to be no supporting evidence, for its visible microscopical detail is in every respect identical to that of preceding telophases. On the contrary, when there is no interkinetal rest it is possible to trace the origin of the paired threads of the heterotype prophases back to the longitudinally split chromosomes of the preceding archesporial telophase. This is irrefutable evidence on behalf of the view that the *paired threads of the heterotype prophases are homologous to those of the somatic prophases*; that is to say, that each thread represents *half* a univalent chromosome of the preceding telophase. Having established this fact, the subsequent phases form a logical story.

During synapsis the lateral association in pairs of the threads is consummated. Whereas in somatic prophases the threads come together to form the univalent spireme filament which segments directly into univalent chromosomes, in the heterotype prophases this univalent spireme filament as it issues from synapsis is continuous, the univalent segments (filaments) being joined end to end, and their separation is postponed until a considerably later stage.

It was not until the success in fixing was attained that all stages in the conjunction in pairs of *entire* univalent lengths of spireme (i.e. of filaments) were observed throughout the phases between post-synapsis and second contraction. In *Osmunda* these stages are not only difficult to fix sufficiently well to show this phenomenon, but they are also quickly passed

[1] See foot-note, p. 156.

through, and consequently may be easily missed. Evidently neither Strasburger (17), Grégoire (10), nor Yamanouchi (19), were aware of them. The first indication of conjunction is to be seen in the drawing together of portions of the univalent loops (filaments) or univalent lengths of spireme filament in the hollow spireme stage. This is also coupled with the connecting together of the approaching spireme filaments by fine transverse strands. The spore mother-cell nuclei of some sporangia may show no signs whatever of this early conjunction, whilst in others it is exceedingly evident. At the approach of second contraction the conjoining becomes more intimate. This phenomenon, again, may be easily overlooked. It is not a case of all the portions of spireme filament in a nucleus conjoining simultaneously, but of isolated examples of conjunction of lengths and segments. During second contraction, the side-to-side conjunction becomes so close that, as the bivalent segments come out of the chromatic aggregation, they appear to split apart. This disjunction of the bivalents bears so close a resemblance to fission, that, if the intermediate conjoining stages had been missed, it would naturally be inferred that this is the opening out of the fission present in the post-synaptic spireme. Apparently, in some instances, univalent segments may achieve the necessary conjunction without entering second contraction, for it is not uncommon to find fully organized bivalent chromosomes lying outside, and independent of, second contraction. Moreover, in some species of *Primula* (4) and *Smilacina* (14), in which the conjunction of univalents (i.e. of filaments) in the post-synaptic stages is extraordinarily clear, second contraction is omitted, and close conjunction in pairs of the univalent segments (filaments) proceeds freely within the nucleus; and when this is accomplished, the univalents separate to form the typical bivalent chromosome.

Although, as a general rule, complete conjunction of univalents (i. e. of filaments) appears to require close lateral conjoining of the filament segments, yet it seems certain that bivalency may be achieved by a less intimate combination, such as a looping over of univalents (filaments), or an end-to-end connexion.

There are no controversial phases from the heterotype diakinesis onwards to the end of the homotype division.

Of recent years Lawson (14) and Nothnagel (16) have both published valuable evidence in favour of the telosynaptic theory. Lawson has found a very clear type of conjunction of univalent segments of spireme in *Smilacina* (14, Pl. I, Fig. 16), and, as in *Primula*, second contraction is omitted. He writes that 'up to the time of this lateral pairing of the spiremes . . . I have been unable to recognise any fundamental difference from the series of changes which occur in the early stages of an ordinary somatic mitosis' (p. 611).

Nothnagel (16) very lucidly describes a telosynaptic series of events in

the pollen mother-cells of *Allium tricoccum*. She traces the parallel threads of the heterotype prophases, which she calls daughter halves of single somatic chromosomes, as originating from the vacuolization of the chromosomes in the preceding telophase of the last division of the sporogenous tissue. The continuity can be followed during the interkinetal rest when the remains of the chromosomes of the telophase are visible as ladder-like structures. During synapsis the parallel spiremes approximate, resulting in a single thick spireme. Apparently she did not see the stages of conjunction. She is of opinion that the radiating loops of second contraction represent somatic chromosomes joined end to end, and that they separate at the outer end. Thereupon an arm of a loop pairs with, and twists round, an arm of a neighbouring loop; thus a bivalent is formed by A + B. No such proceeding has been seen in *Osmunda*.

Summary.

1. For the convenience of readers a glossary of terms consistently used throughout this paper is here given.

'*Thread*' = the longitudinal *half* of an entire univalent spireme or chromosome.

'*Filament*' = an *entire* univalent spireme, i.e. the spireme resulting from the parallel association of two half univalent spiremes or threads.

'*Strands*' = very fine strands of linin (1) connecting the several chromosome segments of early telophase; (2) transversely connecting (*a*) the individuals of a pair of associating and dissociating threads, (*b*) the individuals of a pair of conjoining and disjoining filaments.

'*Association*' = the coming together in pairs, side by side, of two threads or *half* univalent spiremes to form the *entire* univalent spireme or filament, which becomes the univalent chromosome.

'*Fission*' = (1) the longitudinal separation of the entire univalent spireme into two threads or half univalent spiremes; (2) the longitudinal separation of a univalent chromosome into two daughter chromosomes.

'*Conjunction*' = the coming together in pairs of two *entire* univalent spiremes or filaments to form the bivalent spireme which becomes the bivalent or heterotype chromosome.

'*Disjunction*' = (1) the separation of the bivalent spireme into two entire univalent spiremes, or (2) the separation of the bivalent or heterotype chromosome into two entire univalent chromosomes.

2. *Archesporial Divisions.*

The archesporial divisions of *Osmunda* are straightforward, and conform to the scheme generally described for the somatic mitosis of plants.

These phases clearly demonstrate the dual nature of each univalent chromosome, which presumably persists throughout the cycle of chromosome dissolution and reconstitution (see Text-fig., Nos. 1–8).

In late anaphase the substance of each chromosome is seen to be divided already into longitudinal halves—the 'threads' (Pl. VIII, Fig. 2). As the newly formed nucleus proceeds towards rest, the chromosome halves or threads become beaded (Fig. 4). The threads gradually separate widely, and the beads become resolved into finer and finer granules until a fine reticulate state is reached—the so-called resting stage (Fig. 6).

During prophase the series of events is reversed, and the chromosome halves, or threads, reassociate to build up the univalent chromosome. The first indication of the coming together of the halves consists in the drawing together, in parallel pairs, of the fine beaded threads (Fig. 11). Each beaded thread gradually concentrates into spireme (Fig. 16), and the association of the two halves (i. e. the two threads) becomes increasingly intimate until it results in the organization of the completed univalent chromosome (Fig. 21). The space between the associating halves (threads) forms the future line of fission which splits the chromosome into its daughter halves on the equatorial plate.

The daughter chromosomes proceed to their respective poles (Fig. 24), and in late anaphase (Fig. 2) each divides again by fission into longitudinal halves (threads), thus completing the cycle.

3. *Interkinesis between the Telophase of the Last Archesporial Division and the Heterotype Prophase.*

The telophase of the last archesporial division (Pl. VIII, Fig. 25) is indistinguishable from that of the preceding archesporial telophases, and shows the longitudinal splitting of the chromosomes into threads (see Text-fig., Nos. 9–12). The late telophase (Fig. 29) may pass through an interkinetal rest (Pl. IX, Fig. 34), or it may proceed directly into the heterotype prophase (Fig. 36) (see Text-fig., Nos. 13 and 14).

Those nuclei which pass through an interkinetal rest show the same gradual formation of a reticulum by the spreading out of the threads and the fragmentation of the chromatic granules, as in the archesporial divisions. Those nuclei that proceed directly from telophase into the heterotype prophase show transitional stages in which both telophase and prophase characters are present (Figs. 30, 32, and 33). There is no complete separation of the threads or chromosome halves, but traces of paired spireme and paired beads persist from the one phase to the other. These, therefore, substantiate the identity or homology existing between the parallel threads of the telophase (Pl. VIII, Fig. 25) derived from the longitudinally split chromosomes, and the parallel threads of the heterotype prophase

(Fig. 37, &c.), and accordingly establish the nature of each thread of a pair to be that of a *half* univalent chromosome.

4. *First Meiotic Division.*

Osmunda conforms to the *telosynaptic* view of the origin of the heterotype chromosomes (see Text-fig., Nos. 15–27).

The paired spireme threads of the heterotype presynaptic prophases are homologous with those of the somatic prophases; that is to say, each thread of a pair is derived from the longitudinal half chromosome of the preceding telophase. During the phases leading to synapsis, these halved univalent spiremes (threads) become arranged in closely parallel pairs (Figs. 40, 41, &c.), and the individual halves of each pair are identical. Close association of the two individual halves (threads) of each pair is achieved during synapsis. All stages in the preparation for, and in the realization of, association are to be found. Those cases are particularly striking in which the loops of spireme threads are polarized towards one side of the nucleus in synapsis, and association in pairs of the sides of several loops (i.e. of threads) is seen to be taking place simultaneously (Figs. 47 and 48).

The entire filament as it emerges from synapsis is wholly of univalent nature; no unassociated threads or halved filaments remain (Fig. 49). There may be a space in the substance of the univalent spireme between the two recently associated threads; this space marks the future line of that delayed fission which will take effect on the homotype spindle when the daughter univalent chromosomes separate from each other during the homotype mitosis.

After the spireme filament has become unravelled, and is distributed throughout the nuclear cavity, segments of the entire (i.e. made up of the joined halves) univalent spireme filament proceed to conjoin in pairs. The first indication of this conjunction is to be seen in the drawing together of the sides of loops or lengths of spireme filament, the two being joined by a fine transverse strand (Fig. 54, &c.). This conjunction in pairs of univalent spiremes (i.e. of filaments) is prepared for, and more or less achieved, during the stages leading to second contraction (Pl. X, Figs. 68, 69, &c.), but it is finally consummated during second contraction itself (Figs. 71 and 72). In the early conjoining stages, the conjoining filament segments describe figures closely resembling the future heterotype chromosomes (Fig. 65). The two individual filament segments of each conjoining pair are precisely similar (Fig. 69).

As the bivalent segments come out of second contraction they proceed to disjoin and give rise to the typical heterotype chromosome figures (Fig. 74, &c.).

The two entire univalent chromosomes of each bivalent combination separate on the heterotype spindle (Pl. XI, Fig. 82). As the univalent

chromosomes proceed to the poles, fission almost completely separates them into the daughter chromosomes which will separate on the homotype spindle (Fig. 84).

In anaphase (Fig. 85) these daughter univalent chromosomes persist (see Text-fig., Nos. 25 and 26), and as telophase advances each daughter chromosome further splits into two threads (Fig. 86). This apparently additional secondary fission may be accounted for by the fact that *entire* univalent chromosomes, instead of *half* univalent chromosomes, have passed to the spindle poles, as in all other spindle figures.

The daughter chromosomes become resolved into paired beads and parallel threads (Figs. 87 and 88), and the identity of the chromosomes is lost to view.

5. *Second Meiotic Division.*

The homotype prophase follows speedily on the heterotype telophase, and with no intervening rest. The threads derived from the splitting of the daughter halves of the univalent chromosomes of the heterotype anaphase come together in pairs, and concentrate into chromatic masses (Fig. 89). These are in excess of the reduced number of chromosomes, and this is only to be expected, since double the reduced number of chromosomes split apart in anaphase, owing to the splitting of the daughter univalents (see Text-fig., Nos. 25–28).

Throughout the homotype prophase the daughter chromosomes are more or less separate, and they pass on to the spindle as individual entities (Fig. 94) (see Text-fig., Nos. 29–35). As the daughter univalent chromosomes proceed to the spindle poles, each one becomes almost completely divided into longitudinal halves (threads) (Fig. 95). *This is the reappearance of that fission seen in the daughter chromosomes of the heterotype telophase* (Fig. 86). The fission closes up temporarily during the homotype anaphase (Fig. 96), and then reappears during telophase, thus splitting the chromosomes into halves (threads) (Fig. 98). These threads are at first homogeneous and then become beaded. The parallel threads gradually diverge from one another (Fig. 99) and form a reticulum, thus giving rise to the resting stage of the several tetrad nuclei (Fig. 100).

6. The exact correspondence of the two spiremes as they come together in pairs is exceedingly striking. This phenomenon is characteristic, not only of the associating pairs of threads or *half* univalent spiremes of the somatic and presynaptic prophases (Pl. VIII, Figs. 11, 14, 16; Pl. IX, Figs. 41, 46, &c.), which combine to form the *entire* univalent spireme filament, but also of the conjoining pairs of filaments or *entire* univalent spiremes of the post-synaptic stages which unite to form the bivalent spireme (Pl. X, Fig. 69). In both cases the paired spiremes are taut and strained, and the spireme of each pair is precisely similar to its fellow, even to the beading. If their previous history had not been traced, the spiremes

in both instances would be considered to be splitting asunder instead of approaching each other.

7. In the same sporangium nuclei in widely separated stages may occur (Pl. XII, Fig. 101), testifying to the rapidity with which the nuclear phases are passed through. Sporangia have been found in which some of the spore mother-nuclei are in pre-second contraction, whilst others are in diakinesis (Fig. 101); again, some of the nuclei may be in second contraction, while others are in metaphase.

8. Several cases of synangia have been found, one in archesporial division (Fig. 102); the others have their spore mother-cells in the first meiotic division (Figs. 103, 104, 105, and 106). The nuclei of one side of the synangium may be in a considerably more advanced stage than those of the other (Figs. 105 and 106).

Chief Results of the Foregoing Investigation.

1. It is shown that telophasic events have an important bearing on the interpretation of the succeeding prophase.

2. That the sequence of events in prophase can only be interpreted in the light of the preceding telophase.

3. The above-mentioned facts have been found to be of fundamental importance in elucidating the early stages of the heterotype division.

4. In the heterotype division the prophasic stages, ordinarily included under 'synapsis', do *not* consist in the lateral conjunction of *two entire* somatic chromosomes, but in the lateral reassociation of the *threads* in pairs which together make a *single entire* somatic chromosome.

5. Conjunction in pairs of *entire* somatic chromosomes occurs in the stages leading up to, and is finally consummated during, second contraction.

In conclusion I wish to express my great indebtedness to Professor Bretland Farmer, F.R.S., for his most valuable advice and criticism, and also for his kindness in affording me facilities which have rendered the completion of this investigation possible.

The Botanical Laboratory,
Imperial College of Science and Technology.

Bibliography.

1. Bower, F. O. (1900): II. Studies in the Morphology of Spore-producing Members. No. IV. The Leptosporangiate Ferns. Phil. Trans. Roy. Soc., B., cxcii, pp. 29–138.
2. Derschau, M. von (1909): Beiträge zur pflanzlichen Mitose: Centren, Blepharoplasten. Pringsh. Jahr. wiss. Bot., Leipzig, xlvi, Heft 1, pp. 103–18.
3. Digby, L. (1910): The Somatic, Premeiotic, and Meiotic Nuclear Divisions of *Galtonia candicans*. Ann. of Bot., xxiv, No. 96, Oct., pp. 727–57.
4. ——— (1912): The Cytology of *Primula kewensis* and of other related Primula Hybrids. Ann. of Bot., xxvi, No. 102, April, pp. 358–88.

5. DIGBY, L. (1914): A Critical Study of the Cytology of *Crepis virens*. Archiv f. Zellforschung, Leipzig, xii, 1. Heft, pp. 97–146.
6. FARMER, J. B. (1912): Telosynapsis and Parasynapsis. Ann. of Bot., xxvi, No. 102, April, pp. 623–4.
7. ——— and DIGBY, L. (1910): On the Cytological Features exhibited by certain Varietal and Hybrid Ferns. Ann. of Bot., xxiv, No. 93, Jan., pp. 191–212.
8. ——— and MOORE, J. E. S. (1905): On the Meiotic Phase (Reduction Divisions) in Animals and Plants. Quart. Journ. Micr. Sc., xlviii, Part 4, Feb., pp. 489–557.
9. FRASER, H. C. I., and SNELL, J. (1911): The Vegetative Divisions in *Vicia Faba*. Ann. of Bot., xxv, No. 100, Oct., pp. 845–55.
10. GRÉGOIRE, V. (1907): La Formation des Gemini hétérotypiques dans les végétaux. La Cellule, Louvain, xxiv, 2e fasc., pp. 369–420.
11. GUIGNARD, L. (1898): Le Développement du Pollen et la réduction chromatique dans le *Naias major*. Archiv. d'Anat. Micros., Paris, ii, pp. 455–509.
12. HUMPHREY, J. E. (1894): Nucleolen und Centrosomen. Ber. deut. bot. Gesellsch. Berlin, xii, pp. 108–17.
13. ISHIKAWA, M. (1916): A List of the Number of Chromosomes. Bot. Mag., Tokyo, xxx, No. 360, pp. 404–48.
14. LAWSON, A. A. (1912): A Study in Chromosome Reduction. Trans. Roy. Soc., Edinburgh, xlviii, Part iii, No. 25, pp. 601–27.
15. MEVES, F. (1907): Die Spermatocytenteilungen bei der Honigbiene (*Apis mellifica*, L.), nebst Bemerkungen über Chromatinreduktion. Archiv f. mikr. Anat., Bonn, lxx, 3. Heft, Juli, pp. 414–91.
16. NOTHNAGEL, M. (1916): Reduction Divisions in the Pollen Mother-cells of *Allium tricoccum*. Bot. Gaz., Chicago, lxi, No. 6, pp. 453–76.
17. STRASBURGER, E. (1900): Ueber Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. Hist. Beiträge, Jena, iv–vi, pp. 1–224.
18. WILSON SMITH, R. (1900): The Achromatic Spindle in the Spore Mother-cells of *Osmunda regalis*. Bot. Gaz., Chicago, xxx, No. 6, pp. 361–77.
19. YAMANOUCHI, S. (1910): Chromosomes in *Osmunda*. Bot. Gaz., Chicago, xlix, No. 1, pp. 1–12.

EXPLANATION OF PLATES VIII–XII.

Illustrating Miss Digby's paper on the Archesporial and Meiotic Mitoses of *Osmunda*.

All the figures were drawn with a camera lucida under a 2 mm. apochr. Hom. imm. Zeiss N.A. 1–40 with comp. oc. 18 × about 2250.

For the photomicrographs on Plate XII I am indebted to Mr. W. B. Randles, B.Sc.

Figs. 1–24. Archesporial Divisions.
Figs. 25–34. Interkinetal Stages.
Figs. 35–88. First Meiotic Division.
Figs. 89–100. Second Meiotic Division.
Fig. 101. Sporangium containing nuclei in widely separated stages (photomicrograph).
Figs. 102–6. Synangia (photomicrographs).

Osmunda palustris. Figs. 4, 16, 19, 25, 26, 27, 28, 62, 63, 64, 65, 79.
O. palustris var. *undulata*. Figs. 22, 60, 61, 67, 68, 69, 71, 72, 73, 74, 102, 103.
O. regalis. Figs. 32, 48, 82.
O. palustris var. *aurea*. All the remaining figures.

ABBREVIATIONS.

1. *Fixatives*. A.A. = Acetic Alcohol; H. = Hermann; S.C. = Strong Chromic; S.F. = Strong Flemming; S.M. = Strong Merkel; W.F. = Weak Flemming.

2. *Areas of Nucleus*. E. = East; N. = North; NW. = North-West; S. = South; SE. = South-East; SW. = South-West; W. = West.

PLATE VIII.

Fig. 1. *O. palustris* var. *aurea*. Polar view of anaphase of archesporial mitosis. Fission not visible in the chromosomes (S.F.).

Fig. 2. Early telophase showing the splitting of the chromosomes into longitudinal halves or threads (S.F.).

Fig. 3. The nucleus is bounded by a limiting membrane. Some of the individual split chromosome segments are still recognizable, while others have lost their visible identity owing to the separation of their beaded halves (threads) (S.F.).

Fig. 4. *O. palustris*. The threads become resolved into fine beads producing a cloudy effect (W.F.).

Fig. 5. *O. palustris* var. *aurea*. The beaded threads tend to separate from one another, thus inaugurating a reticulum. In some of the chromosome segments the halves (threads) are still closely parallel (S.F.).

Fig. 6. The beaded threads have completely separated, and the nucleus shows a faint reticulum, the chromatin being mostly concentrated in the nucleoli. This is the so-called 'resting' stage (S.F.).

Fig. 7. Very early prophase. The nucleus has a generally more active appearance, and a few large chromatic granules are present in the reticulum (S F.).

Fig. 8. The granules of the reticulum become more distinctly chromatic (S.F.).

Fig. 9. The chromatic granules incline to cluster and are of very varied sizes; the meshes of the reticulum become wider (S F.).

Fig. 10. Some of the threads of the reticulum break down, and the meshes open out, and thus, very gradually, the reticulum becomes converted into a spireme thread. At this stage there are indications of the fine spireme threads tending to arrange themselves in parallel pairs. This is the first sign of the reassociation of the threads, i. e. the longitudinal halves of the chromosomes which separated during the preceding telophase (Fig. 4, &c.). Therefore each thread is of a *half* univalent nature. As prophase advances the reassociation becomes closer until the mature univalent chromosome is organized (S.F.).

Fig. 11. The beaded linin threads form striking parallelisms, as shown in the pair lying horizontally in the E. of the nucleus. Cf. this reassociation of the chromosome halves with the separation of the chromosome halves, as shown in Figs. 4 and 5. Note that the two approaching threads are precisely similar even to the correspondence of the beads (S.F.).

Fig. 12. The linin threads become increasingly distinct and the chromatic beads show more definitely a parallel arrangement, and are often disposed in groups of four (S.F.).

Fig. 13. A slightly later stage; the chromatic beads are somewhat larger (S.F.).

Fig. 14. The chromatin from the beads gradually infiltrates the linin threads, causing them to stain chromatically (S.F.).

Fig. 15. A superficial section of a nucleus from the same sporangium as Fig. 14, showing some of the lengths of associating spiremes as fine parallel threads, whilst others are chromatic (S.F.).

Fig. 16. *O. palustris*. This nucleus shows the close association in pairs of the threads, and the segregation of these paired individual segments suggestive of the future univalent chromosomes. Note the exact similarity of the approaching sides (H.).

Fig. 17. *O. palustris* var. *aurea*. Note the marked association of the threads (S.F.).

Fig. 18. Superficial section of a portion of a nucleus from the same sporangium as Fig. 17, showing that on the close association of two threads the resulting filament becomes considerably thickened (S.F.).

Fig. 19. *O. palustris*. A later stage in which there is an almost complete approximation of the two parallel threads, and the space between them may now be distinguished as the line of *fission*. The spireme filaments curve and undulate, and many of the fine connecting transverse strands have disappeared (A.A.).

Fig. 20. *O. palustris* var. *aurea*. The spireme filaments lengthen out, but are still undulating. In this particular nucleus fission is evident (S.F.).

Fig. 21. Spindle fibres appear and converge towards the nucleus from four points (S.F.).

Fig. 22. *O. palustris* var. *undulata*. The nuclear limiting membrane disappears, the chromosomes concentrate, and vestiges only of fission are to be seen (S.M.).

Fig. 23. *O. palustris* var. *aurea*. Oblique view of a metaphase. The chromosomes are thick and concentrated and show no fission (S.F.).

Fig. 24. The daughter chromosomes show no fission as they proceed to their respective poles (S.F.).

Fig. 25. *O. palustris*. Telophase of the last archesporial division before the heterotype prophase. The chromosome segments are splitting apart into halves (threads); each thread may be more or less homogeneous, or beaded (S.M.).

Fig. 26. The separation of the chromosome halves or threads has proceeded slightly farther; note the clear spaces round the nucleoli (S.M.).

Fig. 27. Superficial view of a portion of a nucleus at this stage; fine strands join the severally paired segments, the skeletons of the former chromosomes (S.M.).

Fig. 28. Very late telophase. The chromosomes are now no longer recognizable owing to the dispersal of their halves, although parallel fine threads are still to be seen. The more aggregated appearance of the chromatin is due to the fixative used (S.C.).

Fig. 29. *O. palustris* var. *aurea*. Typical late telophase showing the spaces round the two nucleoli, the threads having completely separated and fragmented into beads (S.F.).

Fig. 30. A nucleus showing both telophase and prophase characters, evidently passing directly from the telophase of the last archesporial division into the heterotype prophase (see also Figs. 31 and 32). The beaded parallel threads and the presence of three nucleoli are suggestive of telophase, whilst the large size and generally active appearance of the nucleus denote prophase (S.F.).

PLATE IX.

Fig. 31. Superficial section of a nucleus in the same stage as Fig. 30 showing strikingly paired threads (S.F.).

Fig. 32. *O. regalis*. Another nucleus which cannot be definitely described as either in telophase or in prophase. Although actually only one pair of parallel beaded threads is to be seen (in the S. of the nucleus), yet there are indications of parallel arrangements in the groupings of the beads and in the disposition of the fine linin threads (A.A.).

Fig. 33. *O. palustris* var. *aurea*. A transitional nucleus passing directly from telophase of the last archesporial division into the heterotype prophase without an intermediate rest. The nucleus is larger than in rest (Fig. 34) and is active in appearance and stains sharply. Three nucleoli are present; the chromatic beads are larger than those of early prophase (Fig. 36); parallel arrangements of beads and threads are to be seen. It is impossible to designate such a nucleus as being either in telophase or in prophase (S.F.).

Fig. 34. The resting stage between the last archesporial division and the heterotype prophase. The nucleus shows a colourless reticulum with the chromatin concentrated in the nucleoli (S.F.).

Fig. 35. Very early prophase; note the somewhat more sharply staining reticulum and the few chromatic granules (S.F.).

Fig. 36. A nucleus definitely in prophase. The reticulum is more defined, consisting of fine threads with chromatic beads at the junction of the meshes. A few of the linin threads tend to run parallel to one another, and the granules to group themselves in pairs and fours (cf. Fig. 10). This is the first indication of the reassociation of the chromosome halves (threads) which separated in the preceding telophase (Figs. 25, 26, and 27) (S.F.).

Fig. 37. The nucleus has enlarged considerably. The reticulum gradually opens out to form beaded threads, and these threads tend to run in pairs, each thread representing *half* a univalent chromosome of the preceding telophase. As the nuclei proceed towards synapsis the pairing of these *half* univalent spiremes (threads) preparatory to their close association to form the *entire* univalent spireme (filament) becomes increasingly conspicuous (S.F.).

Fig. 38. The linin threads become chromatic and hence definitely spireme in character (S.F.).

Fig. 39. The spireme shows a very striking pairing of its lengths (S F.).

Fig. 40. The pairing becomes more accentuated, and in some parts has resulted in the close association of the two threads to form the entire univalent spireme or filament (S.F.).

Fig. 41. Note the lengths of paired threads connected at intervals by paired beads. There is a slight withdrawal of the nuclear contents in the NW. portion of the periphery preparatory for synapsis (S.F.).

Fig. 42. The spireme thread retreats from the nuclear periphery and is thrown into loops which lie free in the nuclear cavity; towards the margins parallel arrangements are visible (S.F.).

Fig. 43. A superficial section of a nucleus in the same stage as Fig. 42, showing two associating lengths of spireme thread twisting over one another (S.F.).

Fig. 44. Another superficial section of the same stage showing threads running closely parallel (S.F.).

Fig. 45. Early synapsis. Association of the sides of the loops (i. e. of two threads) can be seen towards the margins of the knot, especially in the S. It will be seen that the two threads are separate for a length and then closely associated (S.F.).

Fig. 46. Superficial section of a portion of a nucleus in early synapsis, showing striking similarity of the approaching threads (H.).

Fig. 47. Synapsis showing the polarization of the threads as loops towards one side of the nucleus, and the association of the threads, i. e. the sides of the loops, in pairs actually taking place. This is the consummation of the association of the *half* univalent spiremes (threads) in pairs to form the *entire* univalent spireme (filament) (S.F.).

Fig. 48. *O. regalis.* This nucleus in synapsis is at a slightly later stage to that of Fig. 47. The association of the threads has become closer, leaving only a vestige of 'fission' between them (A.A.).

Fig. 49. *O. palustris* var. *aurea.* The spireme filament is beginning to come out of synapsis. The association is completed; the emerging spireme filament is thick, being the product of the coming together of the two *halves* of univalent spireme, i. e. of two threads. From henceforth the spireme filament is of an *entire* univalent nature. Fission is visible in parts (H.).

Fig. 50. Superficial section of a nucleus coming out of synapsis. Every portion of the spireme filament shows fission and that of a very beaded character, characteristic of Hermann fixation (H.).

Fig. 51. Early hollow spireme. The spireme filament has come out of synapsis and is distributed throughout the nucleus. Fission separates more thread-like sides characteristic of Strong Flemming fixation (S.F.).

Fig. 52. Superficial section of a nucleus in the same stage as Fig. 51, showing the looping of the spireme (S.F.).

Fig. 53. The spireme loops incline to arrange themselves round the nucleolus. In this nucleus fission is still evident, whilst in some nuclei in the same sporangium (cf. Fig. 59) it is closed (H.).

Fig. 54. Figs. 54–8 are studies of the conjunction in pairs of lengths of filament, i. e. of entire univalent spiremes, the first indication of the evolution of the heterotype chromosomes. Note in the loops pointing S. in Fig. 54 the sides are drawn in towards one another and connected by a fine transverse strand. Also the two filaments pointing NW. are connected by fine cross strands (H.).

Fig. 55. Note the conjunction of the univalent sides of the loop suggestive of a future U-shaped chromosome. Fission in the univalent sides, separating the threads, is evident (H.).

Fig. 56. Shows the conjunction of univalents (i. e. of filaments) prophetic of a future X-shaped chromosome (S.F.).

Figs. 57 and 58 show a looping over and conjunction of univalents forming figures typical of heterotype chromosomes. In Fig. 57 only vestiges of fission are to be seen (S.F.).

PLATE X.

Fig. 59. This nucleus is from the same sporangium as Fig. 53, but is in a slightly later stage. Fission has closed, and there is a marked conjunction of filaments, as seen in the looping over of the spireme, and in the S. of the nucleus fine transverse strands join the univalent sides (H.).

Fig. 60. *O. palustris* var. *undulata.* The spireme in the N. portion of the nucleus is beginning to aggregate preparatory for second contraction. Note the conjoining pair of filaments in the SE. attached to the nucleolus. They are separate for a distance except for a fine transverse connexion, and then closely conjoined and then again separate. No fission is visible (H.).

Fig. 61. Note the conjunction of filaments end to end to form a closed ring in the N. of the nucleus, and the twisting over of two filaments in the centre of the nucleus (H.).

Fig. 62. *O. palustris.* The filaments are withdrawing from the nuclear periphery and collecting towards the centre in preparation for second contraction. Note the loop to the S. of the nucleus showing fission. At this stage the spireme filament has definitely segmented and free ends are to be seen (H.).

Fig. 63. Nucleus preparing to go into second contraction. Note the striking example of conjunction in the SW. portion of the nucleus. The filaments are closely conjoined for a length and then diverge to form a closed loop. No fission is visible. The apparently thickened spireme of the nucleus is due to the strong chromic fixative. Towards the SE. striae are visible in the cytoplasm, the first sign of the origin of the spindle fibres (S.C.).

Fig. 64. Superficial section of a nucleus showing the conjunction of filaments. The resulting figures are extraordinarily like those of fully organized heterotype chromosomes (cf. Fig. 78) except for the slightness of the filaments. Fission is visible in the substance of some of the filaments, in others it is closed (H.).

Fig. 65. Another superficial section of the same stage as Fig. 64. Note the bivalent segment on the SE. side showing fission in the independent filaments or segments of univalent spireme, and then their conjunction to form a closed ring showing no fission (H.).

Fig. 66. *O. palustris* var. *aurea*. A study of two conjoining filaments twisting over one another, showing fission in their free ends (H.).

Fig. 67. *O. palustris* var. *undulata*. A nucleus going into second contraction, showing little sign of conjunction. This nucleus is taken from the same sporangium as Figs. 68, 69, and 71, and even from the same microscopic field as Figs. 69 and 71. In Fig. 69 conjunction is advanced, in Fig. 71 the nucleus has entered second contraction (H.).

Fig. 68. As the filaments go into second contraction they show close conjunction in pairs. The nucleus has decreased considerably in size (H.).

Fig. 69. This nucleus is taken from the same sporangium and even from the same microscopic field as Fig. 67. Whereas in Fig. 67 the filaments are relatively thick and show little sign of conjunction, in Fig. 69 close conjunction in pairs has taken place between most of the segments. Note the taut and strained appearance of the conjoining filaments and their precise similarity even to the beading (cf. the same phenomenon in the associating threads, Figs. 16, 41, and 46) (H.).

Fig. 70. *O. palustris* var. *aurea*. A nucleus going into second contraction. In the centre the filaments have collected to form a dense mass. Note the variety in shape, size, and degree of concentration in the filaments not involved in the central mass; some have conjoined and some are in the act of conjoining (H.).

Fig. 71. *O. palustris* var. *undulata*. This nucleus is taken from the same sporangium and from the same microscopic field as Figs. 67 and 69. The greater part of the nuclear contents are in second contraction, the two outlying filaments show a twisted conjunction (H.).

Fig. 72. Another figure of second contraction. Some of the outstanding bivalent segments show a wide separation of their filaments and others close conjunction (H.).

Fig. 73. A nucleus coming out of second contraction. As the bivalent segments emerge some show close conjunction of their univalents, whilst others show stages in the process of disjunction. Above the nucleolus on the E. side is an instance of a univalent spireme emerging in detached portions which speedily join up end to end (H.).

Fig. 74. This nucleus is an example of the second contraction apparently loosening directly into fully organized bivalent chromosomes. The two bivalent segments on the W. side of the nucleus are disjoining (H.).

Fig. 75. *O. palustris* var. *aurea*. In this type of nucleus the bivalent segments come out of second contraction as two closely conjoined univalents, and their disjunction closely resembles fission. The univalent sides as they disjoin are slight, but speedily concentrate (S.F.).

Fig. 76. As the univalent chromosomes disjoin they concentrate and stain homogeneously. Note the thick bivalent segment running N. and S. which has not yet disjoined into its univalent segments (S.F.).

Fig. 77. On coming out of second contraction, the chromosome segments pass to the periphery of the nucleus. Some of the bivalent or heterotype chromosomes are clearly individualized. Fission, which is rarely visible at this stage, can be seen in the univalent segment of the chromosome lying against the S. boundary of the nucleus (H.).

Fig. 78. The chromosomes concentrate rapidly. Note the univalents twisting over one another in the E. of the nucleus, recalling the twisting over of univalent spiremes (i.e. of filaments) in the early conjunction stages (cf. Fig. 64, the segment in the S. of the nucleus) (S.F.).

Fig. 79. *O. palustris*. Spindle fibres make their appearance from four points in the cytoplasm, and the nuclear limiting membrane disappears (S.C.).

PLATE XI.

Fig. 80. *O. palustris* var. *aurea*. The chromosomes return towards the centre of the nucleus. Fission is sometimes visible in the substance of the univalent chromosomes (H.).

Fig. 81. The spindle fibres invade the nucleus, and the chromosomes concentrate considerably and fission closes (H.).

Fig. 82. *O. regalis*. Metaphase of the first meiotic division. The quadripolar spindle has become bipolar (S.F.).

Fig. 83. *O. palustris* var. *aurea*. Polar view of an equatorial plate showing twenty bivalent chromosomes (H.).

Fig. 84. As the entire univalent chromosomes proceed to the poles fission widely separates each chromosome into daughter chromosomes (H.).

Fig. 85. Polar view of anaphase; each univalent chromosome appears double owing to fission still widely separating the daughter chromosomes (H.).

Fig. 86. A second and new fission appears in each daughter univalent chromosome, splitting it into two threads and giving it a fenestrated or vacuolated appearance (H.).

Fig. 87. The nucleus becomes bounded by a limiting membrane; the splitting apart of the sides (threads) of the daughter chromosomes proceeds, and fine transverse strands join the various threads to one another (H.).

Fig. 88. Telophase of the first meiotic division. The identity of the chromosomes is lost to view and the sides are resolved into paired beads and parallel threads (S.F.).

Fig. 89. The onset of the prophase of the second meiotic division. The chromatic nuclear contents become concentrated in rounded masses lying in the portion of the nucleus remote from the cell-plate separating the daughter nuclei (S.F.).

Fig. 90. The nuclear contents mass together (S.F.).

Fig. 91. Spindle fibres invade the chromatic mass (S F.).

Fig. 92. The chromatic segments which are still somewhat indeterminate loosen out along the fibres (S.F.).

Fig. 93. The chromosome segments become greatly elongated and each appears double, having already split into daughter chromosomes (S.F.).

Fig. 94. Metaphase of the second meiotic division. The daughter chromosomes are completely separated before taking up their position on the spindle (H.).

Fig. 95. As the daughter chromosomes proceed to the poles fission almost completely separates them into threads. This is the opening out of the fission prepared for in the alveolization of the daughter univalent chromosomes in the anaphase of the heterotype division (Fig. 86) (H.).

Fig. 96. Polar view of the anaphase of the second meiotic division. The chromosomes draw together and fission closes (H.).

Fig. 97. The chromosomes separate from one another, and fission once more divides them into longitudinal halves or threads which are at first homogeneous (H.).

Fig. 98. The threads become beaded (H.).

Fig. 99. A nuclear limiting membrane appears. The beaded threads separate, but the beaded skeleton of a few of the chromosomes can still be recognized (H.).

Fig. 100. The resting stage of the tetrad. The beads become distributed throughout the nucleus and thus a fine reticulum is formed (S.F.).

PLATE XII.

(Photomicrographs by Mr. W. B. Randles.)

Fig. 101. *O. palustris* var. *aurea*. Spore mother-nuclei in pre-second contraction and in diakinesis in the same sporangium. × 400.

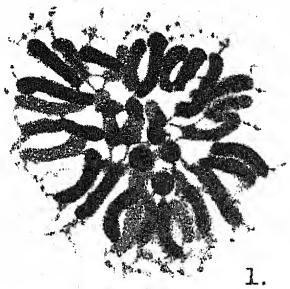
Fig. 102. *O. palustris* var. *undulata*. Synangium in archesporial division. × 350.

Fig. 103. Synangium. The spore mother-nuclei of one side are in synapsis and coming out of synapsis, the nuclei of the other side are coming out of synapsis. × 200.

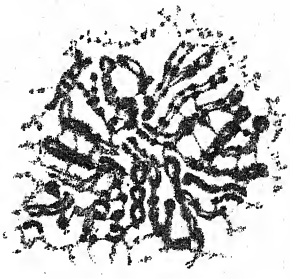
Fig. 104. *O. palustris* var. *aurea*. Synangium. The spore mother-nuclei of the one side are in hollow spireme stage, but as the sporangium lies obliquely the section does not pass through them on the other side. × 200.

Fig. 105. Synangium. The spore mother-nuclei of the one side are in synapsis, and of the other side in diakinesis. × 225.

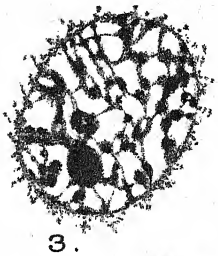
Fig. 106. Synangium. The spore mother-nuclei of the one side are in synapsis, and of the other side in metaphase. × 200.



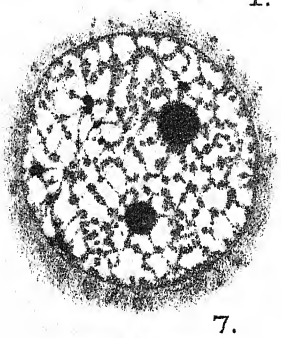
1.



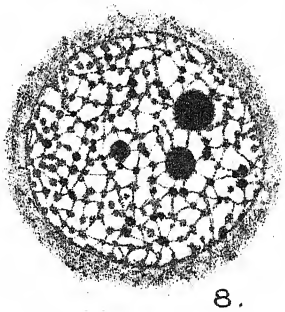
2.



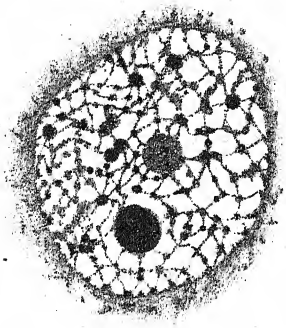
3.



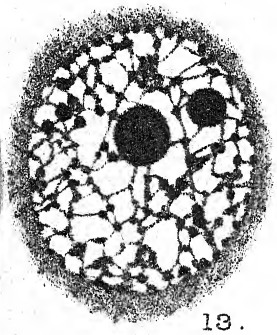
7.



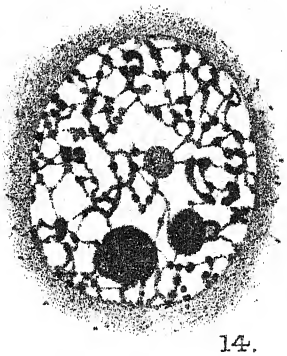
8.



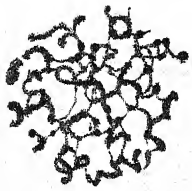
9.



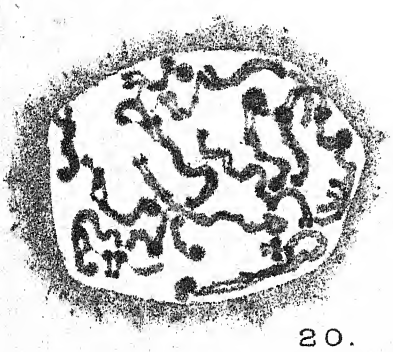
13.



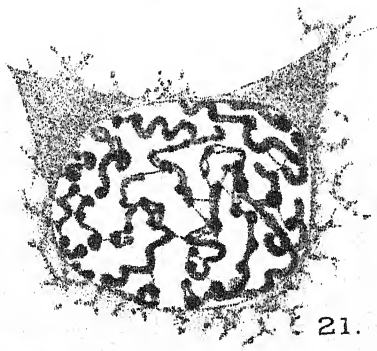
14.



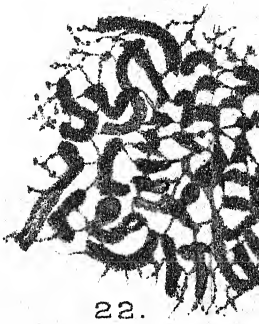
15.



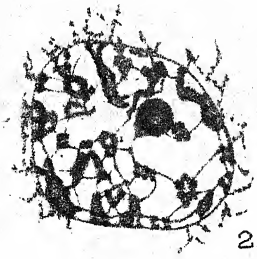
20.



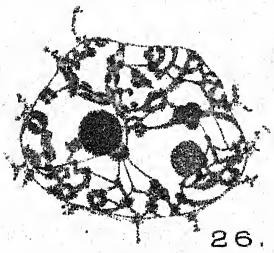
21.



22.

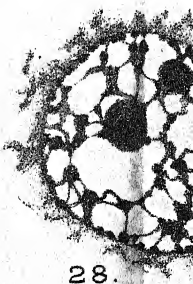


25.

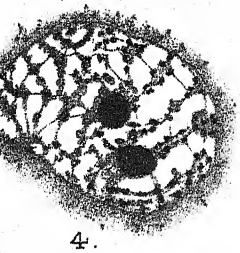


26.

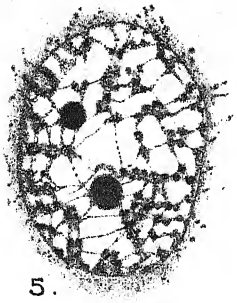
27.



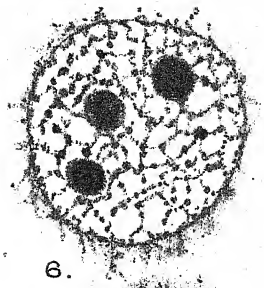
28.



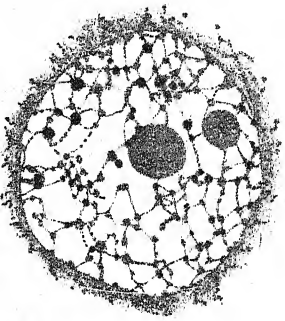
4.



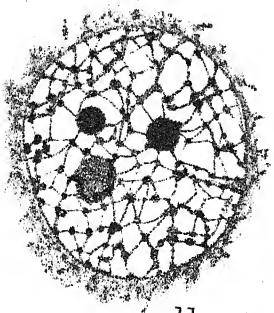
5.



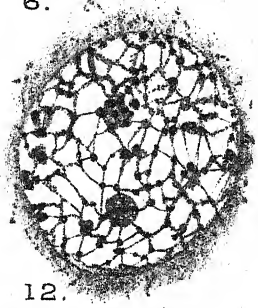
6.



10.

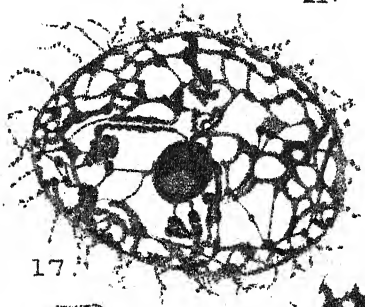


11.

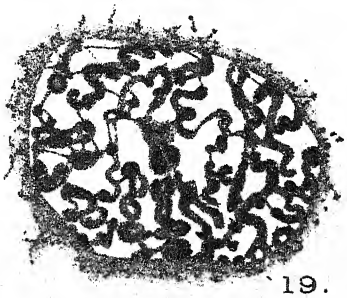


12.

16.



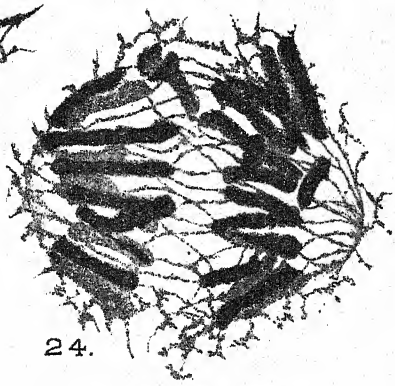
17.



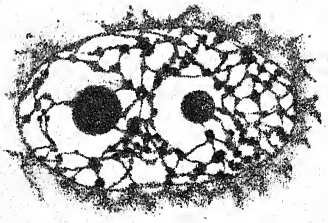
19.

18.

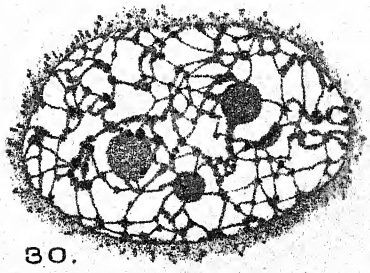
23.



24.

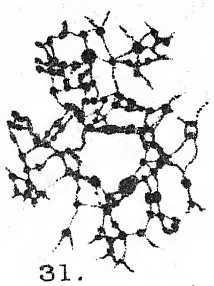


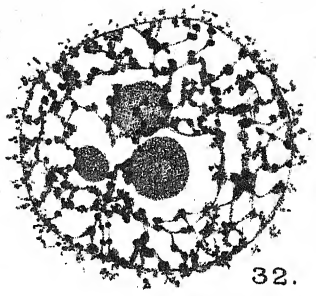
29.

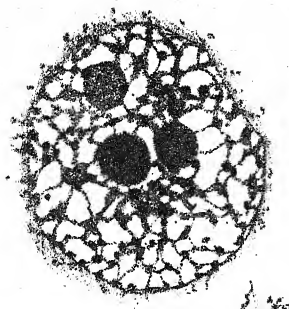


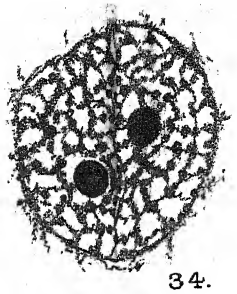
30.

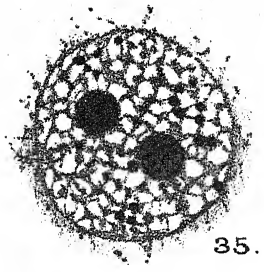
Huth lith. et imp.

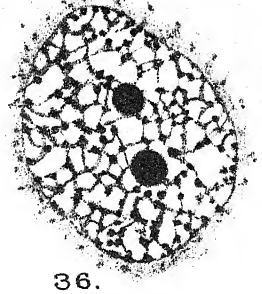

31.

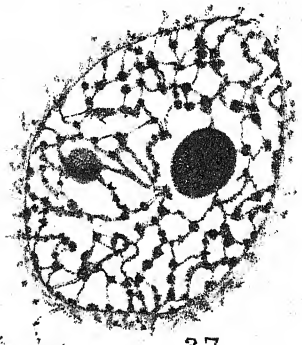

32.

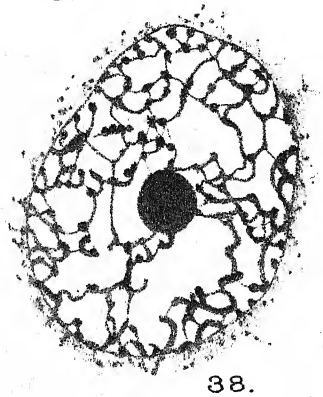

33.

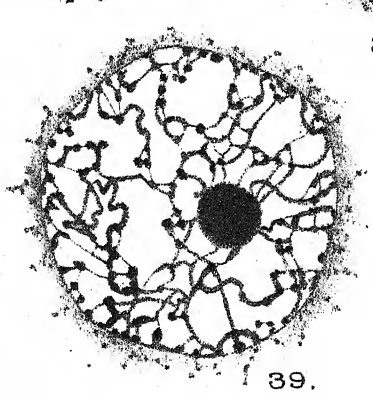

34.

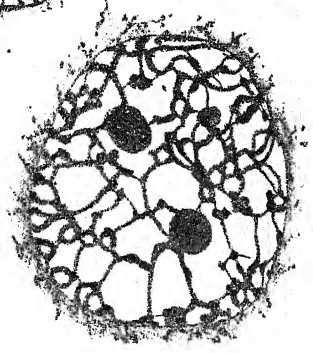

35.

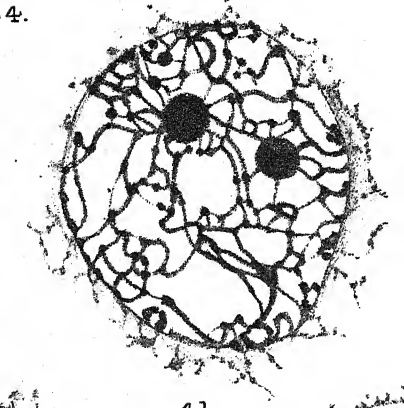

36.

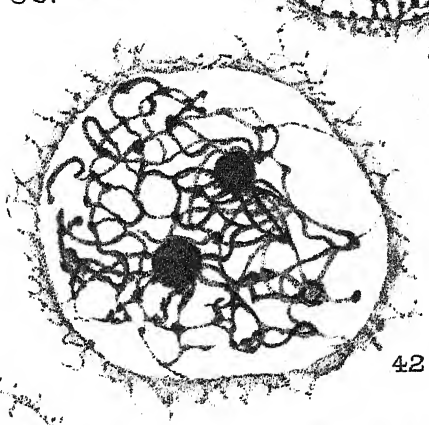

37.


38.

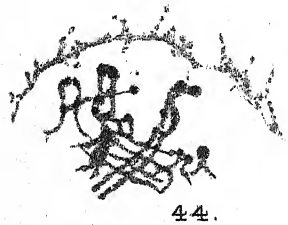

39.

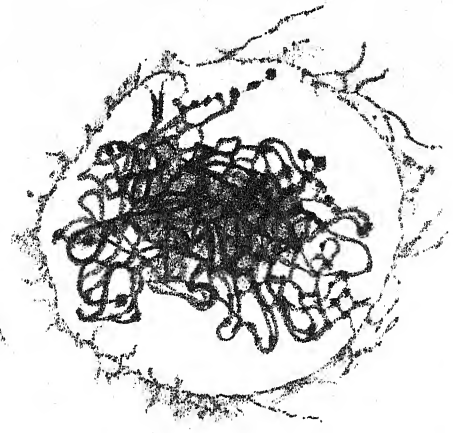

40.

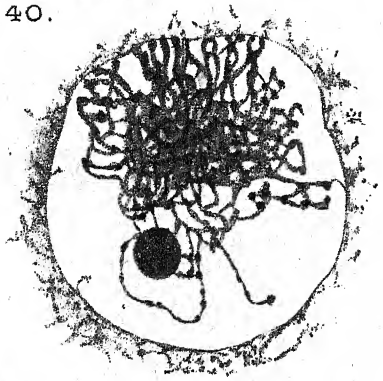

41.

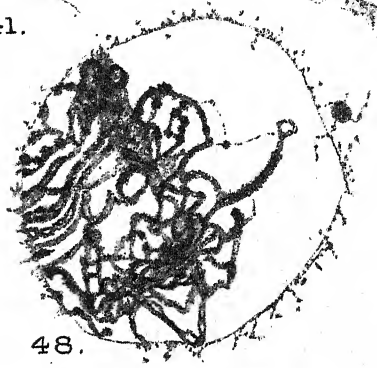

42.

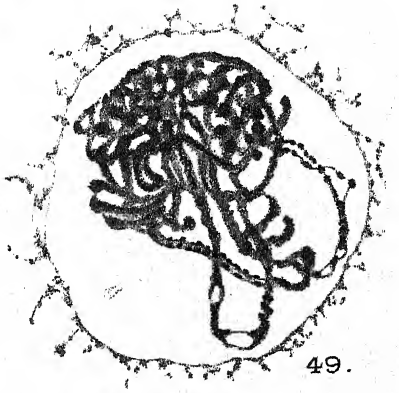
43.

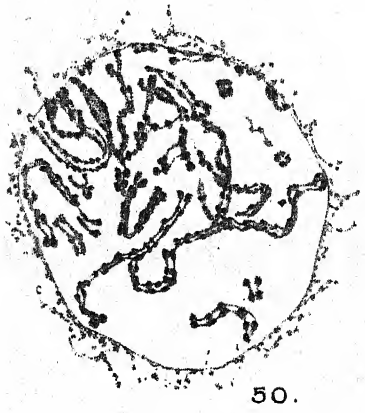

44.

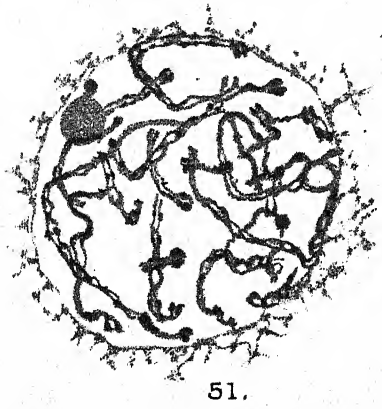

45.


47.


48.

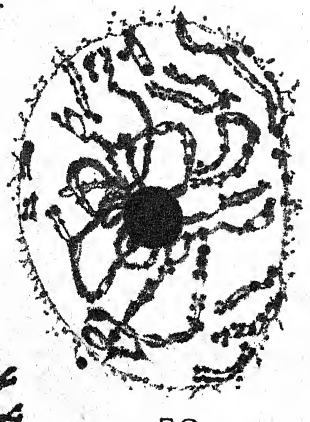

49.

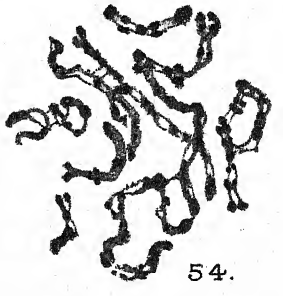

50.


51.

46.

52.

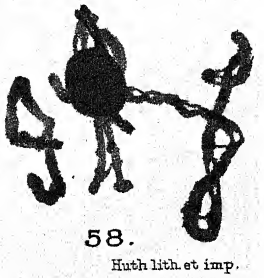

53.


54.

55.

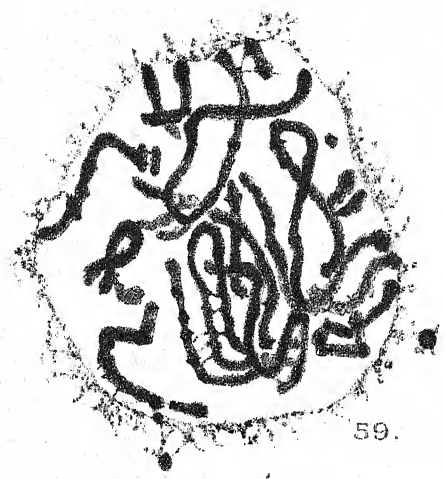
56.

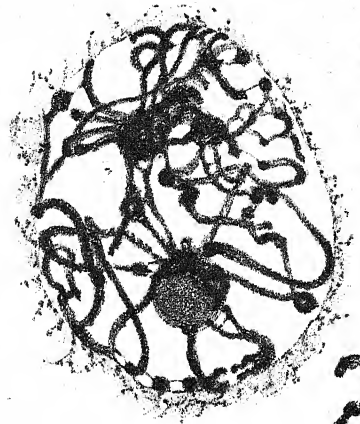
57.

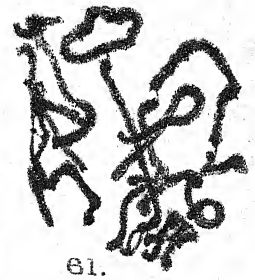

58.

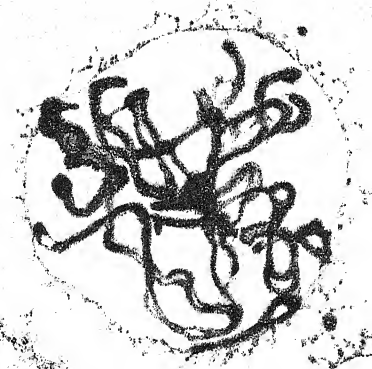
Huth lith. et imp.

DIGBY—MITOSES IN OSMUNDA.


59.


60.

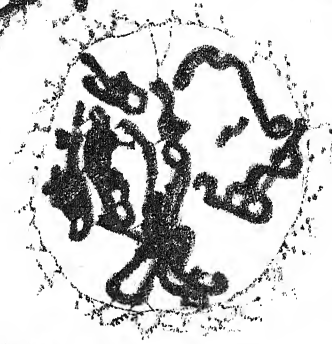

61.

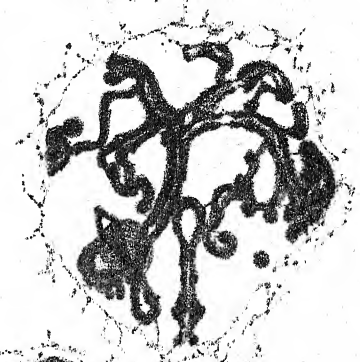

62.

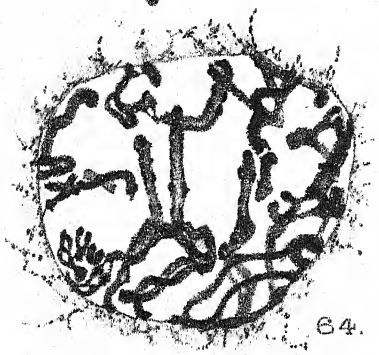
63.

66.

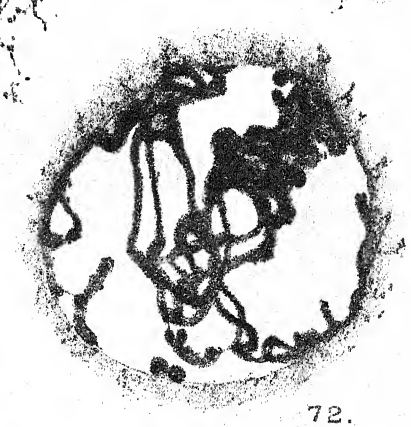
67.

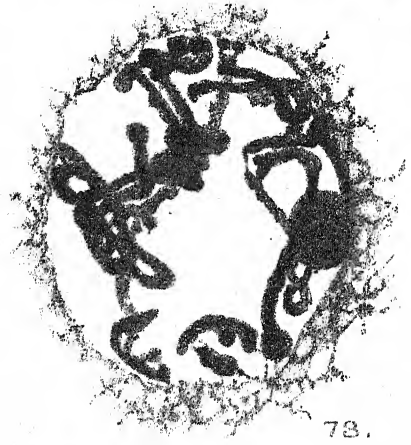

68.


69.

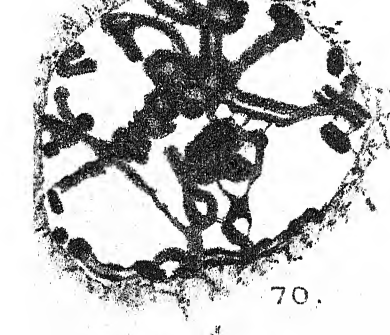

64.

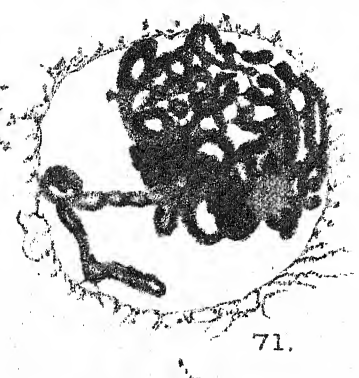
65.

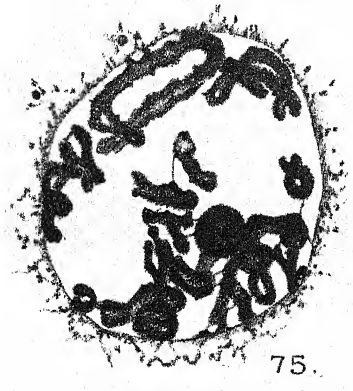

72.

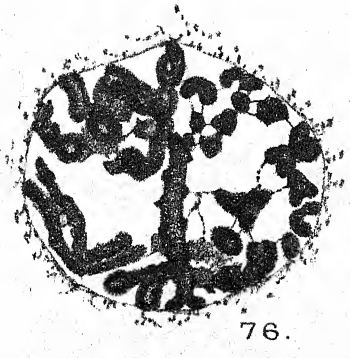

73.

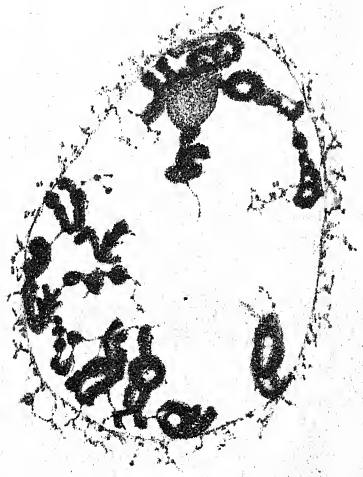
74.

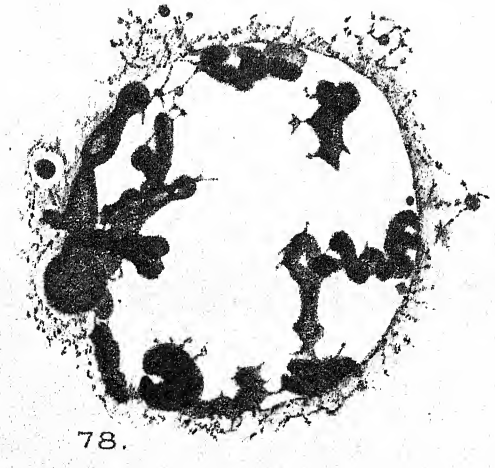

70.

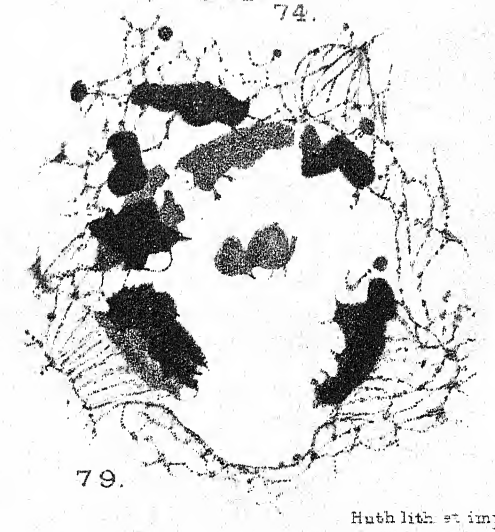

71.


75.


76.

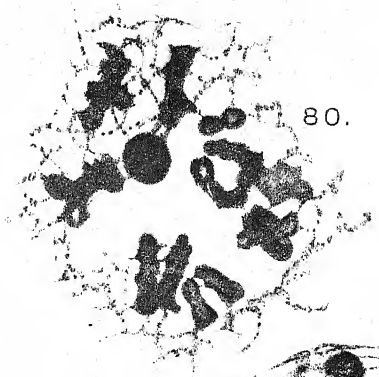

77.

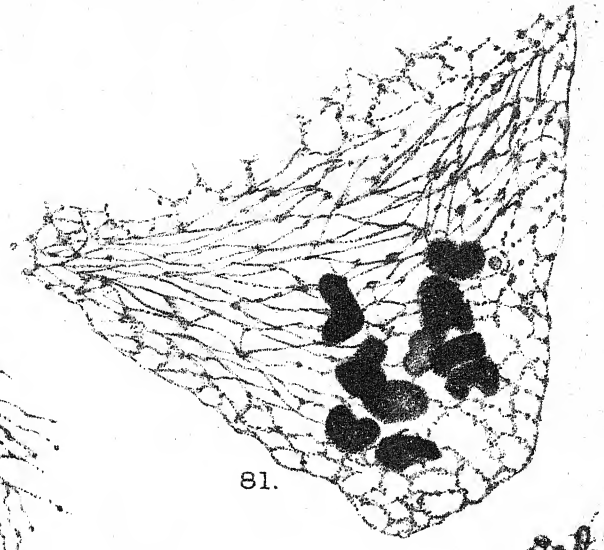

78.

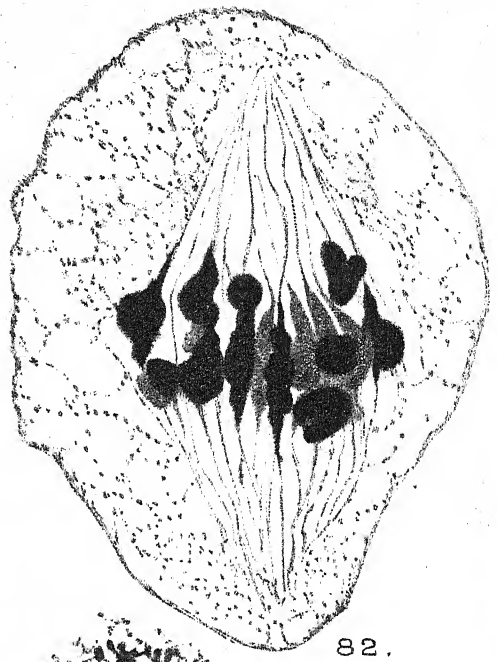

79.

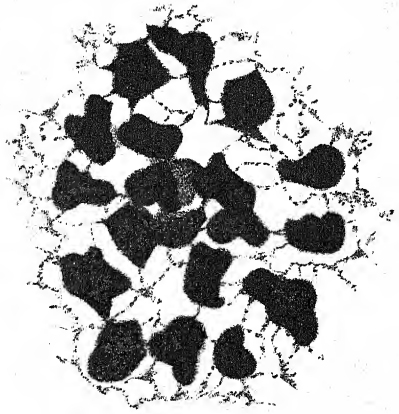
Huth lith. et imp.

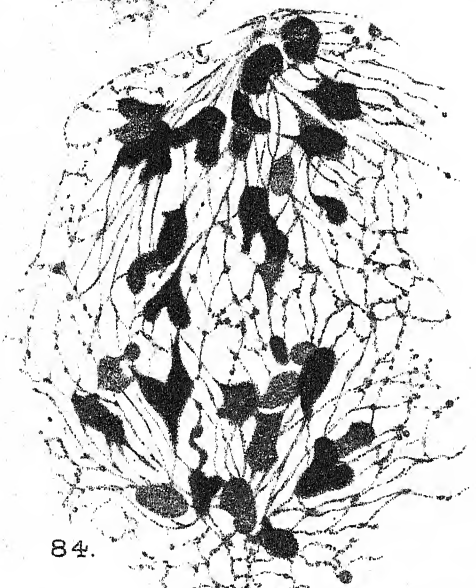
DIGBY — MITOSES IN OSMUNDA.

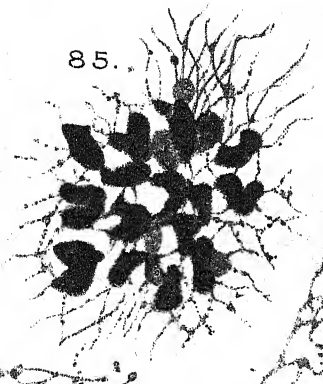

80.


81.


82.

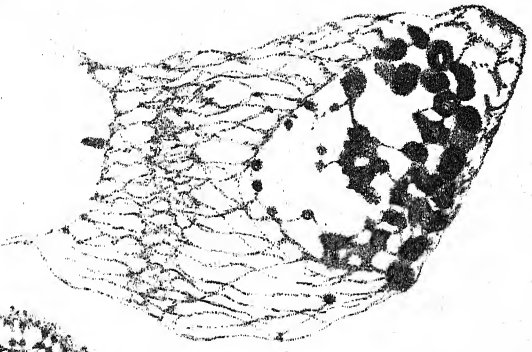

83.

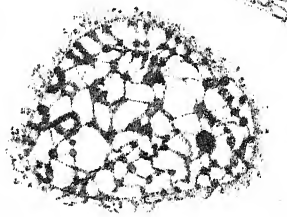

84.


85.

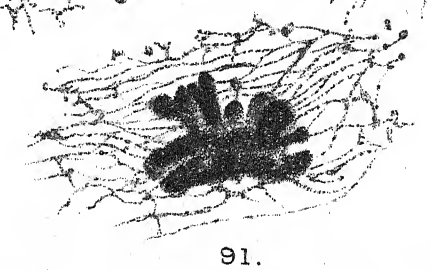
86.

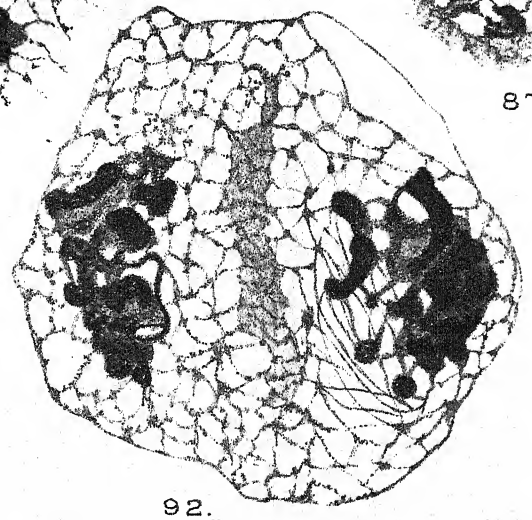
87.

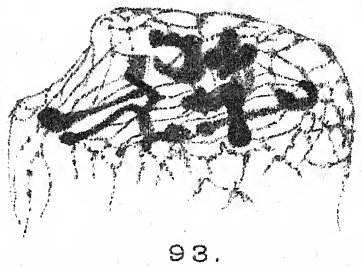

89.

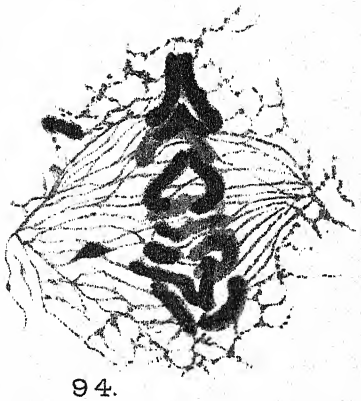

88.

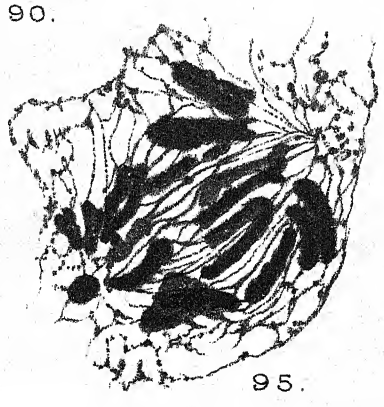
90.

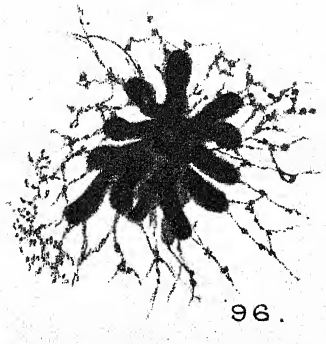

91.

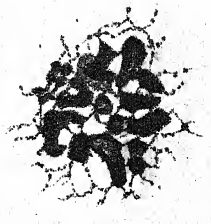

92.

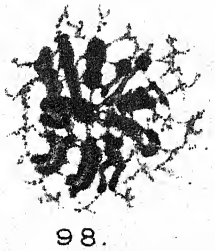

93.

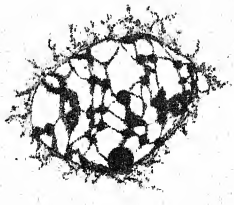

94.

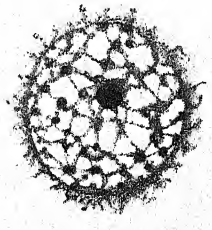

95.


96.


97.

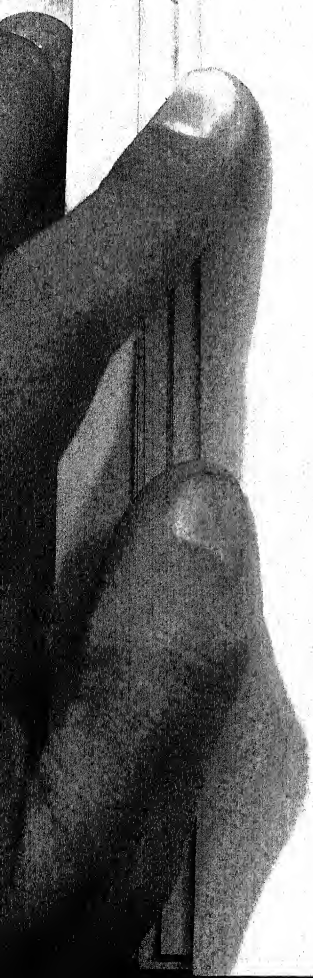

98.

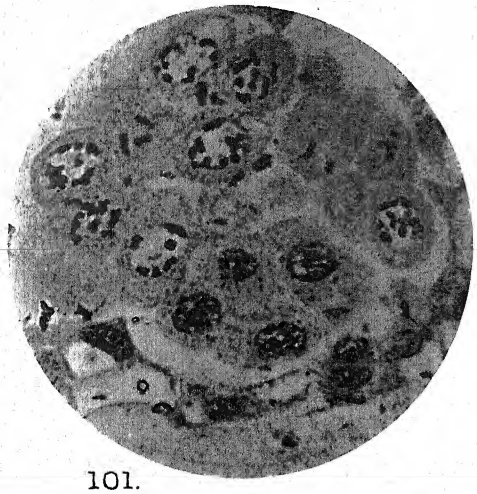

99.


100.

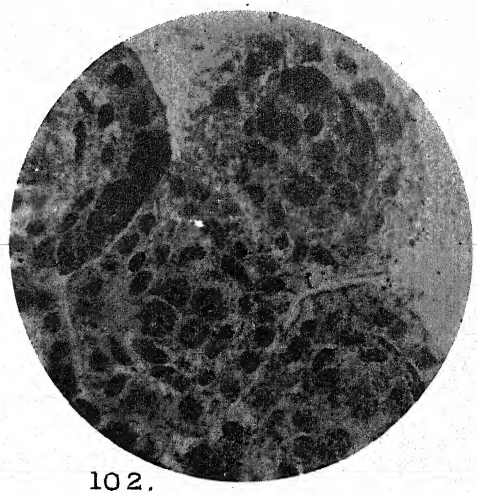
DIGBY—MITOSES

Huth lith. et imp.

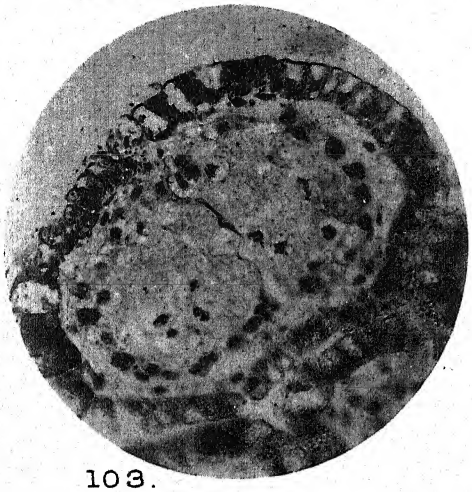




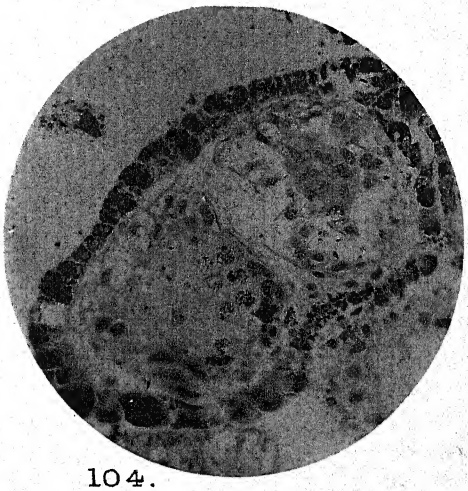
101.



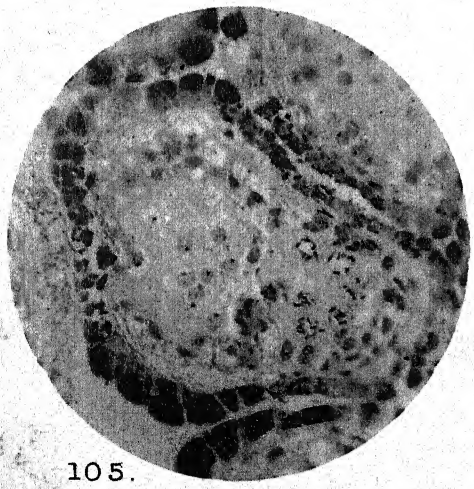
102.



103.



104.



105.

106.

W.B.R. phot. Huth coll.

DIGBY—MITOSES IN OSMUNDA.

Remarks on the Organization of the Cones of Williamsonia gigas (L. & H.).

BY

THE LATE E. A. NEWELL ARBER, M.A., Sc.D., F.L.S.,

Trinity College, Cambridge, University Demonstrator in Palaeobotany.[1]

With five Figures in the Text.

AS is well known, the organization of the cones of *Williamsonia gigas* (L. and H.) has remained a palaeobotanical puzzle since the days when Williamson [2] and Yates [3] first attempted that restoration independently in 1849. The memoirs which bear on this matter and have been published since that date must now approach, if they do not exceed, a hundred in number. A detailed account of these researches, with a full and up-to-date bibliography, has recently been given by Professor Seward,[4] so they need not be enumerated here.

At the present time there is much which is still admittedly obscure in regard to the morphology of the cones of this plant. They have not yet been found with all their organs in continuity, and there seems unfortunately to be little likelihood of such incrustations being obtained in the near future. From analogy with Bennettites, we should expect that the microsporophylls in particular were fleeting, caducous organs, and thus the chance of obtaining specimens fossilized while these structures were mature and before they had been shed appears to be very small indeed. We must look forward rather to the happy discovery of petrified male cones of this or some similar species in the future, a discovery of which we need not despair, seeing that a female petrified cone of *Williamsonia* is now known. Considerable progress has, however, been made in the recognition of what is either the complete or the incomplete female cone, firstly by Lignier,[5] and more convincingly by Seward [6] quite recently. It may be well therefore to

[1] Owing to the author's death before this paper was finally revised, the responsibility for any errors which it may contain rests with me. I have to acknowledge a grant from the Royal Society in aid of the preparation of this and other memoirs left by the author in various stages of completion.—AGNES ARBER.

[2] Williamson (1849).

[3] Yates (1849).

[4] Seward (1917), vol. iii, chapter 37.

[5] Lignier (1903).

[6] Seward (1917), vol. iii, pp. 429, &c.

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

sum up the difficulties which remain in order to see how the position stands at present. This I propose to do briefly here.

The chief uncertainties are as follows:

(1) Were the cones monosporangiate (unisexual) or amphisporangiate (bisexual)?

(2) Where were the male sporophylls attached?

(3) What structure, if any, was borne on the axis of the cone above the female organs (interseminal scales and seeds)?

(4) Was there an infundibular expansion, somewhat similar in form to the united whorl of male sporophylls, but of a sterile nature, and where was it attached?

Were the Cones monosporangiate or amphisporangiate?

On the question as to whether the cones were monosporangiate or amphisporangiate there will always be differences of opinion until the perfect male cone has been discovered. It is, at present, a case merely of the balance of probability. On the amphisporangiate side, the older view, we find ranged the opinions of Lignier,[1] Wieland,[2] and quite recently Seward,[3] who says (1917) 'they may have been bisporangiate—a view that seems to me the more probable—but this has not been demonstrated'.

That the cone of *Williamsonia* was monosporangiate, and that there were separate male and female cones, was first advocated by Nathorst,[4] and more recently has been supported by Thomas.[5] The present writer supports the Monosporangiate theory on the following grounds.

He believes that all the parts of the two cones, male and female, were figured by Williamson[6] as far back as 1870, and that it is merely a matter of piecing the parts together correctly. The illustrations in question are Figs. 1, 2, 4, and 5 of Williamson's Pl. 52, and Figs. 6–8 of the same author's Plate 53. The latter set of specimens are now known to represent the apex of the axis still bearing interseminal scales, probably sterile. More complete specimens of the lower parts of the same cones were figured by Saporta[7] from British specimens in 1891. The only doubt then as regards the female cone is whether any organ was borne at the tip of the axis of that cone at the region of the terminal mamilla, termed by Williamson the corona, a point to be further discussed presently.

As particularly pertinent to this inquiry, emphasis may be laid on a fact, which appears to have been overlooked in recent years. The cones of *Williamsonia* had two quite different axes, exactly as Williamson first figured them, and despite Lignier's[8] opinion that the staminal whorl

[1] Lignier (1907).
[2] Wieland (1911), p. 462.
[3] Seward (1917), vol. iii, pp. 423–4.
[4] Nathorst, (1909) p. 30, (1911) p. 26.
[5] Thomas (1915), p. 137.
[6] Williamson (1870).
[7] Saporta (1891), vol. iv, Pl. 18, Fig. 2; Pl. 19, Fig. 2; Pl. 20, Fig. 2.
[8] Lignier (1907).

occurred on the same axis as the female organs. The cones which we now know to have been partly or wholly female had a long *conical* axis, the best illustrations of which are those of Saporta already referred to above. The shape of these axes is also shown in the restoration of the female cone given here in Figs. 1 and 2. Other cones, however, possessed a flask-shaped or urn-shaped axis as figured on Williamson's Pl. 52, Fig. 4 (refigured in outline here as Fig. 5, p. 177). The writer has also seen more than one other example of the same structures among the specimens of *Williamsonia* at Cambridge. The shape of this axis is entirely different from that of the female flower, and thus there are certainly grounds for very strong suspicion

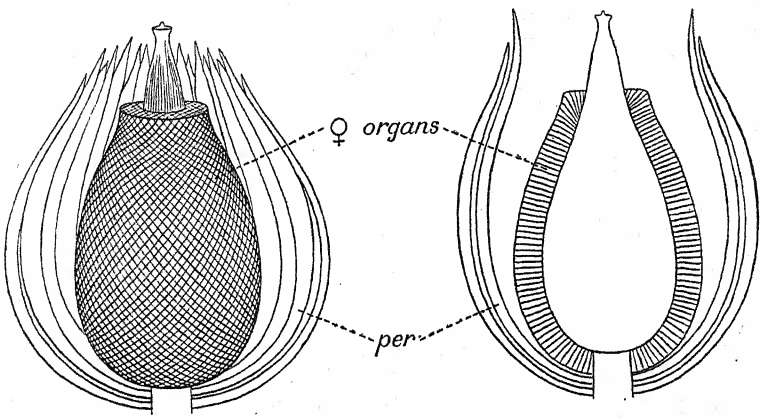


FIG. 1. Restoration of female cone of *Williamsonia gigas* with the front bracts (*per.*) removed (half natural size). FIG. 2. The same in section; ♀ organs = interseminal scales or seeds.

that this plant possessed two cones. None of the urn-shaped axes, regarded by the writer as male, ever show any trace of interseminal scales such as are almost always persistent at the base or apex or both regions of the female flower. Any organs which they bore were clearly attached apically, and it is difficult to imagine that they could have been other than the microsporophylls.

WHERE WERE THE MALE SPOROPHYLLS ATTACHED?

Perhaps the greatest difficulty in regard to the Williamsonian cone is to decide where the male sporophylls were attached. These organs are of course now exceedingly well known as detached objects. It should be remembered in this connexion that Nathorst,[1] to whom we owe our knowledge of these organs in particular, has shown that they were borne *terminally* on something. The axis bearing them was not produced beyond the cup of united sporophylls. That fact is incontestable. The male sporophylls

[1] Nathorst, (1909) pp. 11, 12, (1911) p. 20.

were thus certainly not attached below the interseminal scales. It follows therefore that they were borne either at the apex (corona) of the female conical axes, or on the urn-shaped axis distinguished above. My own view is that the latter possibility is almost certainly correct. If the urn-shaped axes did not bear the microsporophylls, what did they bear? They must have borne some organ beyond doubt. They certainly did not bear interseminal scales, unless in some other more distal region, now missing, and even in that case one would have to admit that the cones of *Williamsonia* were dimorphic.

My view is that Williamson's Plate 52, Fig. 1, was seated on the apex of the axes seen in Figs. 4 and 5 of the same plate, and that his Fig. 2 is

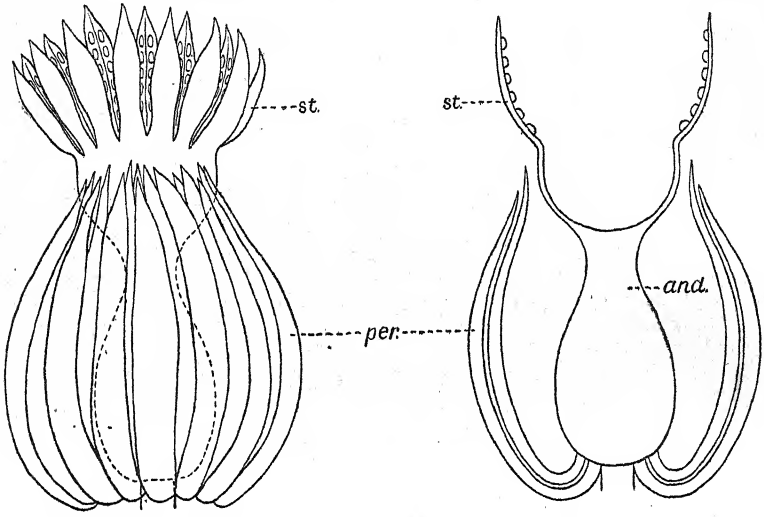


FIG. 3. Restoration of male cone of *Williamsonia gigas* (half natural size). FIG. 4. The same in section. *st.* = whorl of microsporophylls; *per.* = bracts; *and.* = androphore.

simply the lower surface of the cup of united microsporophylls. I therefore restore provisionally the male cone of *Williamsonia* as shown in Figs. 3 and 4.

If this is correct the male strobilus in this species had a distinct gonophore, or more strictly speaking androphore (*and.* in Figs. 3 and 4), whereas the female cone had none. That is to say, there was a considerable elongation of the internode or internodes between the perianth bearing nodes at the base of the cone, and the node bearing the whorl of microsporophylls. Such a gonophore occurs in the case of several Angiospermous amphisporangiate flowers, though somewhat rarely. The genus *Gynandropsis* (family Capparidaceae), of South America and elsewhere, furnishes a well-known example. In *Williamsonia*, the object of the gonophore no doubt was to elevate the microsporophylls when mature out

of the circumscribed space enclosed by the perianth members when the cone was immature.

The male cone of *Williamsonia* is probably not the only strobilus of this group possessing a gonophore. In *Williamsoniella coronata*, recently instituted by Thomas,[1] we find both the male and female organs of this amphisporangiate cone borne on a long stalk. It is true that perianth segments (so-called bracts) are not known to occur at the base of this stalk,

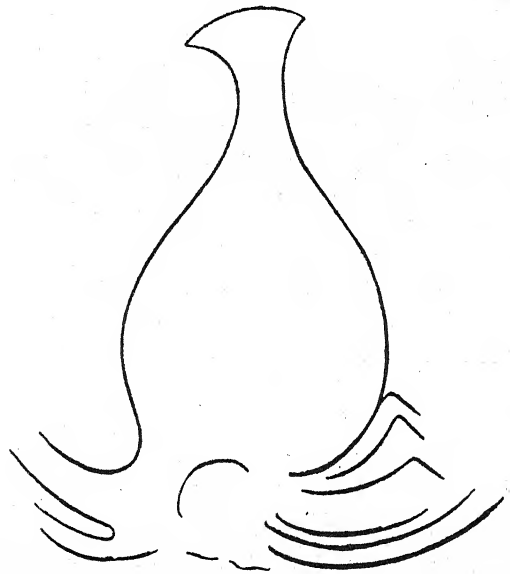


FIG. 5. Outline of the 'pyriform axis' (androphore) of a male cone of *Williamsonia gigas* (after Williamson, W. C., 1870, Pl. 52, Fig. 4), natural size.

but at the same time this organ may be at least provisionally interpreted as being of the nature of a gonophore.

WERE ANY STRUCTURES BORNE AT THE APEX OF THE FEMALE CONE?

The question as to whether any structure was borne at the 'corona' of the female axis must be left open. In *Williamsoniella coronata*, mentioned above, the axis is also prolonged somewhat beyond the region of the interseminal scales, though to a much less degree than in the female *Williamsonia* cone. There is no evidence, however, that it bore any other structure above the female organs.

In the case of *Williamsonia*, I think it is very unlikely that anything was attached in that region. Certainly the male sporophylls were not borne here, and if anything was attached in this region it must have been some other organ. It should also be recalled that many other examples of

[1] Thomas (1915), Text-fig. 1.

female cones of other species of *Williamsonia* are known as impressions, and in one case as a petrifaction. In none of these is there any evidence that the axis projected beyond the zone of the interseminal scales. The female cone of *Williamsonia gigas* appears to be quite exceptional in this respect. It is therefore extremely improbable that any organ at all was attached at the corona.

Was there a Sterile Infundibulum?

The question whether there was a sterile infundibulum may, I think, be almost dismissed. It is an idea which persists as a relic of the times when the nature of the whorl of male sporophylls was misunderstood, and probably the idea arose originally from Williamson's Fig. 2 on his Plate 52. There is very little doubt that the organs in question represent simply the lower surface of the microsporophyll whorl. In this view I agree with Seward,[1] as opposed to Nathorst[2] and Lignier.[3] Williamson himself regarded the specimen mentioned above as simply the other surface of the organ which he illustrated on Fig. 1 of the same plate. If any such organ did exist it is unknown to me, and it could only have been attached at the corona at the apex of the female flower, at which point indeed it has been restored by Lignier.[4]

Conclusions.

While in the absence of continuity between the male organs of the cone of *Williamsonia gigas* (L. and H.) and its axis it is impossible to prove the exact morphology of the fructifications of this plant, I conclude that the balance of probability points as follows in regard to the chief uncertainties which exist as to the organization of the fructifications of this fossil.

(1) The cones were probably monosporangiate.

(2) The female cone possessed a conical axis, sheathed in perianth segments below, and bearing seeds and interseminal scales above. The tip of the axis projected for about 2 cm. beyond the highest interseminal scales, as is also the case, but to a less extent, in *Williamsoniella coronata*. In all probability no other organ was borne on this axis, either at the tip or elsewhere.

(3) The male cone possessed an urn-shaped axis sheathed in perianth segments below. This axis was of the nature of a gonophore. On it was seated apically the whorl of partly united male sporophylls. It did not bear interseminal scales.

(4) There is no evidence of any sterile infundibular organ attached to or terminating either cone. All the organs of these cones have been known since 1870 in continuity, except the male sporophyll whorl and its gonophore.

[1] Seward (1917), vol. iii, p. 428. [2] Nathorst (1909), pp. 12–13. [3] Lignier (1907).
[4] Lignier (1903), Text-fig. 8, p. 35; see also Seward (1917), vol. iii, Fig. 548 on p. 432.

Bibliography.

Lignier, O. (1903): Le Fruit du *Williamsonia gigas* Carr. &c. Mém. Soc. Linn. Normandie, vol. xxi, p. 19, 1903.

———— (1907): Sur un moule litigieux de *Williamsonia gigas* (L. and H.) Carr. Bull. Soc. Linn. Normandie, sér. 6, vol. i, p. 3, 1909 for 1907.

Nathorst, A. G. (1909): Palaeobotanische Mitteilungen. 8: Über *Williamsonia*, *Wielandia*, *Cycadocephalus* und *Weltrichia*. K. Svenska Vetenskap. Handl., vol. xlv, No. 4, 1909.

———— (1911): Palaeobotanische Mitteilungen. 9: Neue Beiträge zur Kenntnis der *Williamsonia*-Blüten. K. Svenska Vetenskap. Handl., vol. xlvi, No. 4, 1911.

Saporta, Le Marquis de (1891): Plantes Jurassiques, tome iv: Types proangiospermiques et Supplément Final. Pal. Francs., sér. 2, Végétaux, Paris, 1891.

Seward, A. C. (1917): Fossil Plants, vol. iii. Cambridge, 1917.

Thomas, H. H. (1915): On *Williamsoniella*, a New Type of Bennettitalean Flower. Phil. Trans. Roy. Soc, Ser. B, vol. ccvii, p. 113, 1915.

Wieland, G. R. (1911): On the Williamsonian Tribe. Amer. Journ. of Science, vol. xxxii, p. 433, 1911.

———— (1916): American Fossil Cycads, vol. ii. Washington, 1916.

Williamson, W. C. (1849): On the scaly vegetable heads or collars from Runswick Bay, supposed to belong to *Zamia gigas*. Proc. Yorkshire Phil. Soc., vol. i, pt. 1, p. 45, 1849.

———— (1870): Contributions towards the History of *Zamia gigas*, Lindl. and Hutt. Trans. Linn. Soc., vol. xxvi, p. 663, 1870.

Yates, J. (1849): Notice of *Zamia gigas*. Proc. Yorkshire Phil. Soc., vol. i, pt. 1, p. 37, 1849.

A New Auxanometer.[1]

BY

F. M. HAINES,

Demonstrator, Botanical Department, East London College.

With two Figures in the Text.

HAVING regard to the slow rate of growth usually obtained under laboratory conditions, and the small magnifications effected by existing auxanometers, an endeavour has been made to design an instrument which will record the growth of the plant on a much larger scale, that is with a much higher 'magnification' than has, as far as the author is aware, ever been attempted hitherto, and which will at the same time allow of the observation extending, if necessary, over a period of at least three or four days. Although the form of apparatus to be described can claim no advantage over its predecessors in the matter of simplicity, the manipulation should present no difficulty after the first setting up, and the care necessary will not seem disproportionate when the fact is taken into consideration that the instrument is capable of magnifying the growth to such an extent that its representative length on the recording graph may be varied at will from 20 to 100 times the actual increase in length of the plant.

An experimental instrument fitted with everything except the compensating gear for the stretching of the fibres has shown itself capable of giving smooth curves on the recording sheet for periods of two days with a magnification of fifty, and has been checked by a Ganong auxanometer working on the same plant; but as, owing to the present conditions, only a rough working model has so far been constructed, the present account must be looked upon as merely preliminary.

The chief features in the new instrument are as follows:

1. The magnification is not produced by concentric pulleys of different radii arranged as in Ganong's instrument, nor by a lever as in Farmer's, but by employing a differential pulley, the plant being (indirectly) connected by

[1] From the Botanical Department, East London College.

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

a fibre to the hook which usually carries the load, while the inking pen is attached to the thread on which the 'work' is done.

2. The inking mechanism, to avoid friction, does not travel on a vertical wire as in Ganong's instrument, but runs horizontally on a mono-rail, two-wheeled trolley.

3. The pen on the trolley travels over a rotating drum which is actuated by a large falling weight and made to revolve once in twelve hours by a clock mechanism; and

4. The particular arrangement of pulleys over which the fibres pass forms a complete compensating mechanism for general expansions and contractions in the fibres, so that no error is introduced by variations in the relative humidity of the surrounding atmosphere.

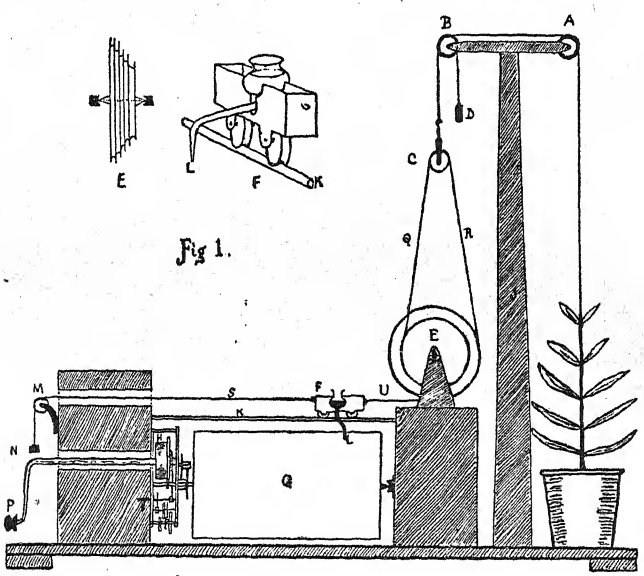


Fig 1.

FIG. 1.

Fig. 1 is a diagrammatic representation of the instrument, showing only the essential features without the compensating gear for changes in length of the fibres. A fibre attached to the tip of the plant round a cotton-wool pad passes over the two pulleys A and B, and down to the hook which carries the pulley C. The pulley C is in the loop of a thread Q R, of which the end R passes round one of the grooves of larger radius of the differential pulley E, while the end Q passes round a groove of smaller radius in the opposite direction. Each end passes about one turn round its own groove and is then affixed to the rim of the pulley. Round that groove on E which has the largest radius passes a fibre U, one end of which is affixed to the pulley, while the other is attached to the trolley F. The

latter runs on two grooved wheels on the single rail K, and carries a glass pen L (see inset, Fig. 1), the point of which rests on the drum G. G revolves once in 12 hours and possesses clips, which are not shown in the figure, for affixing graph paper. As the plant grows the pulley C drops by the same amount as the plant apex rises. Suppose the plant apex rises and the pulley C falls a distance d, then if the thread R passes round a groove on E of radius r_1, Q round a groove of radius r_2, and U round a groove of radius R, it can be easily shown that the trolley F and pen L move along the drum a distance $\frac{2\,d\,R}{r_1-r_2}$. For, if the pulley E turn through one complete revolution, F moves a distance $2\pi R$ (to the left, say), the thread R descends a distance $2\pi r_1$, and the thread Q ascends a distance $2\pi r_2$. C descends a distance equal to half the difference between the distances moved by the threads Q and R, $= \frac{1}{2}\{2\pi r_1 - 2\pi r_2\} = \pi(r_1-r_2)$. The magnification or ratio $\frac{\text{distance moved by F}}{\text{distance moved by C}} = \frac{2\pi R}{\pi(r_1-r_2)} = \frac{2R}{r_1-r_2}$, so that if the plant grow a distance d the pen moves a distance $\frac{2\,d\,R}{r_1-r_2}$. When the plant grows it allows the differential pulley to turn so that the trolley F carrying the pen may move over the drum. The movement is produced by a small hanging weight N, and communicated to the trolley by means of the fibre S which passes over the pulley M. In order that there may be no undue strain on the plant, the weight N is such that it only just exceeds the value of the limiting friction of the trolley and pulleys. With the same object the weight D is provided to counterbalance the weight of C.

As the pen moves along and the drum G revolves, a spiral line is traced out, the pitch of the spiral representing the twelve hours' growth multiplied by $\frac{2R}{r_1-r_2}$. The pulley E is made with five separate grooves of radii 5 cm., 4·9 cm., 4·8 cm., 4·6 cm., and 4·0 cm. (see inset to Fig. 1), so that any desired magnification may be obtained by using appropriate pairs or threes of the grooves. Any of the fibres may be affixed to any of the grooves on E, the end of each fibre being provided with a little hook which fits into a notch on the circumference and so facilitates the operation of altering the magnification. At T is a clock-work mechanism for rotating the drum. It is actuated by a heavy weight (not shown) hanging on the end of a flexible brass wire which passes round a drum at H. The weight is wound up by the handle P. The pulleys throughout are mounted on needle or 'frictionless' bearings.

In the apparatus in its simplest form as described above, it will be noted that any stretching of the fibres due to changes in humidity in the atmosphere all acts in the same direction, namely to make the growth appear *too much*, and as the magnification is large, and there is great length

of fibre, the error introduced would be serious in accurate work. In order to compensate for the variability in the lengths of the fibres the arrangement employed is that seen in Fig. 2. In this figure it will be observed there is no longer a single continuous fibre from the plant apex to the pulley C as in Fig. 1, but there are three distinct fibres X_1X_2, Y, and Z. The fibres X_2 and Y when they reach the pulley A pass round it a few turns and then become attached to its rim. Similarly the fibres Z and X_1 pass round the pulley B a few turns and are then also attached. In each case the two fibres run round the pulley in the same groove and in the same direction, but the pulleys A and B, instead of being simple, as in Fig. 1, are now double, each possessing two distinct grooves of the same radius and side by side (see inset, Fig. 2). In the second groove of each pulley there is attached a fibre running in the opposite direction to the other two, which when it leaves the pulley hangs down and carries a small weight. The weights V, V′ carried on these fibres are exactly equal, and their function, which will be more fully understood later, is to maintain a tension in X_1X_2. The reason that *v* and *v′* run in separate grooves on the pulleys A and B is to avoid friction with the other fibres, and to prevent the possibility of those which are being wound on to the pulleys becoming superposed on those which are leaving. The grooves are figured, for the sake of clearness (in Fig. 2), as if they were of different radii, but this is not actually so, their radii being, as already stated, exactly equal. As in Fig. 1, the pulley C is counterbalanced by the weight D, and, when the plant grows, is allowed to fall by the same distance as the stem apex rises. Now that the nature of the pulleys and connexions is understood it will be readily seen from the figure that stretching in the fibres Y and Z will tend to make the reading *too large*, while stretching in the fibre X_1X_2 will tend to make the reading *too small*. Also stretching in Q R, which in this respect acts like a single fibre of length *x* (see Fig. 2), tends to make the reading *too large*. For compensation, therefore, the length of X_1X_2 must be made equal to the sum of the lengths *x*, Y, and Z. As the growth of the plant and fall

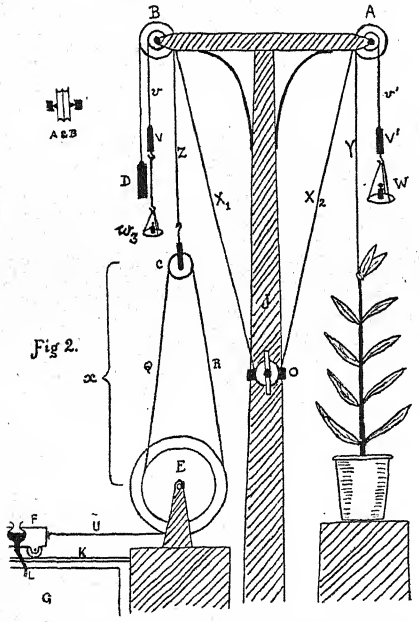


Fig 2.

FIG. 2.

of C are small compared with the length of x, this relation holds sensibly true throughout the experiment.

If the only possible source of error were that introduced by stretching of the threads consequent upon changes in the relative humidity of the atmosphere, this condition would be sufficient in itself to ensure compensation; but as the fibres are also liable to stretch as a result of the tensions to which they are subjected, complete compensation will only be obtained if the average of the tensions in the fibres Z, Y, and (Q + R) taken together be made equal to the tension in the fibre X_1X_2. This condition is secured by giving appropriate values to the weights V, V′ and W (Fig. 2), and these values are determined as follows:

Suppose the tension maintained in the fibre U by the weight $N = t$. Then the tension in Z will be equal to the weight of $C + \frac{2tR}{r_1 - r_2}$, and the tension in $Q = \text{tension in } R = \frac{tR}{r_1 - r_2}$.

As $V = V'$, the tension in X is only due to the weights V, V′, and we have

$$\left.\begin{aligned} T_Y &= W \\ T_X &= V' \\ T_Z &= C + \frac{2tR}{r_1 - r_2} \\ T_{(Q+R)} &= \frac{2tR}{r_1 - r_2} \end{aligned}\right\}$$

where C represents the mass of the pulley C, and T_X, T_Y, &c., represent the respective tensions in the fibres X, Y, &c. For equilibrium a weight w_3 is provided, equal to $\frac{2tR}{r_1 - r_2}$.

For compensation (see above),

$$T_X = \frac{T_Y + T_Z + T_{(Q+R)}}{3}$$

i.e. $V' = \left\{W + C + \frac{2tR}{r_1 - r_2} + \frac{2tR}{r_1 - r_2}\right\} \Big/ 3.$

$= \left\{W + \frac{4tR}{r_1 - r_2} + C\right\} \Big/ 3$, or collecting the constant quantities, R, r_1, r_2, t, and C to one side of the equation, we get

$$3V' - W = \frac{4tR}{r_1 - r_2} + C.$$

With the exception of the value of t, which can be found by preliminary experiment in a manner to be described later, all the quantities on the right-hand side of this equation can be easily found by direct measurement or weighing, so that for perfect compensation a weight W is placed in the scale-pan, which satisfies the above equation using the given values of V and V′. If the tension produced in Y be found insufficient, or great enough to harm the growing point, the weights V and V′ may be altered and the corresponding value for W again found from the equation and placed in the scale-pan. The value of V is of the order five to ten grammes,

and W may be anything from about 1·5 grm. down to 0·5 grm. or less, according to the nature of the plant on which the experiment is conducted.

All stretching of the fibres is now compensated for except that of the fibre U, which connects the trolley and differential pulley. Stretching here is far less serious than elsewhere as it is not magnified by the instrument. It will be seen that, supposing the instrument be magnifying the growth a hundred times, even if the fibre stretch as much as the plant grows (which it is very unlikely to do) the error will only be one per cent., and is therefore scarcely worth correcting for. With lower magnifications and any very accurate work, however, the error may be completely eliminated by using a control fibre. A fibre 10 cm. long attached to a trolley of its own running on a rail by the side of the first may be fixed to a special hook on the stand carrying the differential pulley and be subjected by similar means to the same tension as the first fibre. The expansions and contractions of the control fibre are recorded at one end of the drum, and if they amount to anything appreciable, the correction for the particular length of the fibre U which has run out from E at any moment may be calculated and added or subtracted from the recorded reading as the case may be. In order to facilitate the determination of the length of U a scale of centimetres is provided beside the rail K, the zero-mark being perpendicularly below the bearings of E. When only a twelve hours' observation is taken and the travel of the trolley is large, the paper on the drum, when unfolded, will reveal two graphs which may be added together algebraically to give the correct curve.

Before an experiment, when no plant is connected with Y and the scale-pan W is empty, small weights are gradually added to a light scale-pan hanging at N, until the weight is found which will *just* set the experimental trolley and differential pulley, &c., in motion. Let the weight found $= w_1$. Next the weight is found which sets the trolley in motion when disconnected from the differential pulley. Let this weight $= w_2$. Then, when the whole apparatus is fitted up for an experiment, and the weight in the scale-pan at N is w_1, the tension in the fibre U is given by the equation $t = (W_n + w_1) - (W_n + w_2)$, where W_n is the weight of the scale-pan at N.

$$\text{i.e. } t = w_1 - w_2.$$

This is the value of t which should be substituted in the expression

$$3V' - W = \frac{4tR}{r_1 - r_2} + C,$$

when finding the correct value of W which ensures compensation in the fibres Q, R, X_1X_2, Y, and Z (Fig. 2). With a given value for the weights V and V′, therefore, the complete working equation for W is as follows:

$$W = 3V' - \left\{\frac{4R\,(w_1 - w_2)}{r_1 - r_2} + C\right\}.$$

This, and the equation for the magnification, namely

$$M = \frac{2R}{r_1 - r_2},$$

are the only equations needed in the practical work.

A brief outline will now be given of the method of manipulation of the instrument. The chief points in the preparation for an experiment may be enumerated as follows:

1. Weigh the pulley C.

2. Connect the fibres Q, R, and U with grooves on E which give the desired magnification.

3. Place the plant in position and measure the distance Y from its apex to the pulley A.

4. Find the sum of the lengths x and Z by measuring from the centre of E to the centre of B, and subtracting the length of the mounting of C.

5. Calculate $x + Y + Z$, and move the pulley O until $X_1 + X_2 = x + Y + Z$. (During these operations, pulleys which have loose threads on them may be clamped, if necessary, by special provisions to prevent them being turned by unbalanced weights.)

6. Attach the weights V and V′.

7. Find the weight w_1 with the trolley detached.

8. Find the weight w_2 which turns all the pulleys when the trolley is attached to E. The value of w_2 may be incorrect for two reasons: (*a*) the friction of the pulleys A and B will be less than during the experiment, as the weights W and w_3 are not yet in place, and (*b*) because the friction of the pen L on the drum will be greater than during the experiment as the drum is at rest instead of in motion. As the weights W and w_3 are very small compared with the other weights producing friction in A and B, namely, V, V′, C, and D, the difference in friction values due to their absence will be practically negligible, and the speed of revolution of the drum being also very small, the two errors may be taken as counterbalancing one another. If they do not counterbalance, the calculated value of W will be too small (assuming the errors due to friction in A and B to be the most serious), and W may be increased by a few milligrammes at the beginning of the experiment. This will overcome the friction of A and O, and so restore the slight tension in the thread Y.

9. Calculate the value of t and the tension in Z due to t alone $\left(\text{i.e. } \frac{2tR}{r_1 - r_2}\right)$. Counterbalance this tension by a suitable weight placed in the scale-pan at w_3. Also use the equation to find W. If the value found be suitable, place the weight in the scale-pan; if unsuitable, repeat the operation with a different value for V and V′.

10. Attach Y to the growing point.

11. Adjust O very slightly until F is at the correct end of the drum.

12. Set the drum in motion.

At this point the whole system is in equilibrium, for in the cases of A and B the moments are equal in the two directions and the tension in Z has been arranged to counterbalance exactly the tension in U. When the plant grows the tension in the fibre Y is released so that the moments of the forces tending to turn A to the right become greater than those tending to turn it to the left. A therefore turns to the right and increases the tension in X_2. This increases the tension in X_1 and turns the pulley B to the right, allowing C to fall. The tension in Q R falls so that E revolves to the right as a result of the tension in U. The length of U is therefore increased so that the trolley F moves over the drum. When the trolley reaches the end of its travel it automatically stops the clockwork so that further rotation causes no unnecessary lines on the graph, and the record may be removed at the operator's convenience.

In conclusion, the author wishes to tender his heartiest thanks to Dr. Fritsch and Dr. Salisbury, who have given him every help and facility for the prosecution of this work.

A Comparative Account of the Root-nodules of the Leguminosae.

BY

ETHEL R. SPRATT, D.Sc., A.K.C.,

University of London, King's College, and Battersea Polytechnic.

With Plate XIII and five Figures in the Text.

In the Text-figures *m.* = meristematic zone; *B.* = bacteroidal tissue; *c.* = outer protective cells; *v.b.* = vascular strand; *r.* = root.

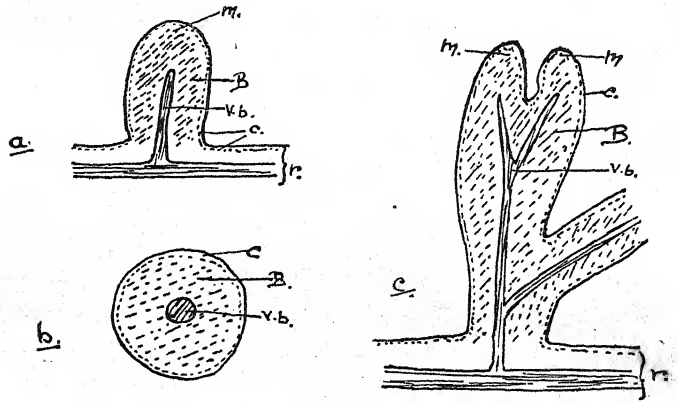
PLANTS which are capable of producing root-nodules when *Bacillus radicicola* penetrates into the root-hairs and subsequently the cortical cells of the root may be placed in two classes—those which belong to the Natural Order Leguminosae and those which do not. All these structures have been shown by several investigators to be actively concerned with assimilation of nitrogen. The bacteria utilize the free nitrogen of the atmosphere, and since the plants associated with them can live in very poor soil they produce nitrogenous material which can be assimilated by the plant.

This symbiotic association is characteristic of the two gymnospermous families Cycadaceae and Podocarpaceae, the dicotyledonous families Elaeagnaceae and Myricaceae, and the genera *Alnus* and *Ceanothus*. Although these plants are widely separated in any other classificatory scheme, they are all non-legumes producing root-nodules containing *Bacillus radicicola*. Each of these nodules has individual characteristics, but in every case the bacteria penetrate the root-hair and enter the cortex of the root without causing any morphological change, until a young lateral root during its passage through the cortex of the parent root becomes infected, when its development is so altered that it becomes a nodule. The plerome was already differentiated when the bacteria entered, therefore they attacked the cortical cells only, the stele retaining its central position and the growing point remaining apical (see Text-fig. 1).

The nodules which are characteristic of the Leguminosae are produced by infection of the root-hairs and cortex by the same organism, which here stimulates the cortical cells to increase in size, thus producing a local swelling which very soon becomes visible (see Pl. XIII, Fig. 6). A basal meristem

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

is differentiated from the pericycle, and in this area one or more vascular strands appear and become continuous with the vascular tissue of the root. By subsequent growth it breaks through the parenchyma of the root and may become quite a large structure, consisting of a mass of central cells containing bacteria, surrounded by a zone of parenchyma in which there are a number of vascular strands, which are either collateral, with the xylem towards the outside and exarch, or concentric, with the xylem central. Both types may occur in the same nodule. The xylem consists of tracheides, usually spirally marked, and parenchyma. The phloem is simply narrow, very elongated cells. Each strand is surrounded by a few very definite layers of cells which form a kind of bundle-sheath.



TEXT-FIG. 1. *a.* Longitudinal section of root and young nodule of *Alnus*. *b.* Transverse section of nodule of *Alnus*. *c.* Longitudinal section of part of old branched nodule of *Alnus*. × 10.

The nodule is protected by a varying number of layers of cells developed from a meristematic layer continuous with the phellogen of the root, which may become dead and empty, or in some cases thickened (see Pl. XIII, Figs. 7, 8, 9).

Whatever its ultimate form and however much it may resemble any particular non-leguminous nodule, e.g. *Vicia Faba* and *Alnus*, the nodules in these two classes are fundamentally different. In the non-legume it is a modified root, whilst in the legume it is an exogenous growth arising in the cortex, which develops a peripheral vascular system and has not a growing point which is primarily differentiated in an apical position.

The leguminous nodules have received a good deal of attention from a large number of investigators. In 1867 Woronin described and figured them, showing the central mass of bacteroidal cells surrounded by an outer layer in which were several vascular bundles. Peirce in 1902 says they are modified lateral roots, but subsequently described their origin from the layer immediately outside the endodermis. They have, however, been very

largely accepted as modified lateral roots, and the organism responsible for their formation has been the main subject of research. It has been variously described as a fungus by Frank in 1879, Marshall Ward in 1887, and others, also as simply proteid matter by Brunchorst, but in 1888 Beijerinck called the organism *Bacillus radicicola*, and showed it to be a true Schizophyte. The formation of infection threads led many to regard the organism as a fungus, but in 1900 Dawson demonstrated that these are not fungal hyphae but masses of little rod-like bacteria, which have divided very rapidly and remained associated together, forming zoogleal threads in which they pass from cell to cell (Pl. XIII, Fig. 7).

It had long been thought that the nodules on leguminous roots were connected with nitrogen assimilation; this was definitely established to be so by Hellriegel and Wilfarth in 1888. A few years later Nobbe and Hiltner and also Mazé demonstrated that it was the bacteria in the nodule which were capable of utilizing the atmospheric nitrogen, and much has since been done in Germany, America, and also in this country in an endeavour to make a wide practical application of this power which the bacteria possess. That the bacteria have a source of nitrogen supply apart from that of the host plant is supported by the statement made by Moore, that the percentage of nitrogen present in the nodule is greater than in any other part of the plant.

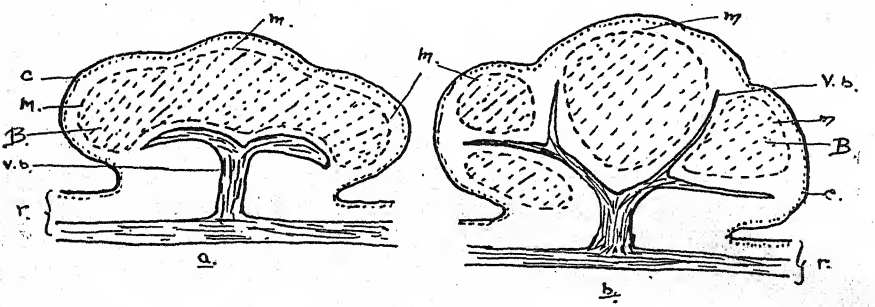
The organism is polymorphic, and different forms occur, not only in connexion with different plants but also in different cells of the same nodule. Nobbe thought each tribe of the Papilionaceae had its own specific organism. This, however, cannot be, because although the organism adapts itself to a particular host and will therefore more readily infect another host of the same kind, in 1901 the American Board of Agriculture found it possible to cross-inoculate any legume from any other except Lupin, and Bottomley has since cross-inoculated Tares with the organisms from *Acacia*, *Alnus*, and *Elaeagnus*, i.e. the two sub-orders of the Leguminosae, Papilionaceae, and Mimosoideae with one another, and a legume with the two non-legumes *Alnus* and *Elaeagnus*. Beijerinck in 1888 said there were two kinds of *Bacillus radicicola*, distinguished by the medium in which they flourished, which was either acid or alkaline. Most legumes do not incline towards an acid soil, but Lupin and the particular race of *Bacillus radicicola* which inhabits the nodules on its roots do, and since in an alkaline soil, or a plant which normally lived in one, the environment would be so very different, it is probable that the adaptation required is too great to be easily obtained. This possibly has a further bearing on the structure of the nodules produced by plants living under very different conditions.

The Natural Order Leguminosae is the second largest family of Phanerogams, having about 440 genera and 7,000 species, living in every kind of soil and climate and showing a variety in habit which includes trees,

shrubs, herbs, and climbers. Amongst so much diversity one naturally looks for differences in the root-nodules of these plants, and they exhibit great variation in form and size, and some also in their anatomy.

From the investigation of a large number of genera it seems possible to place them in four groups, thus:

I. The Genisteae type, in which the nodule is primarily spherical, with a spherical meristem outside the bacteroidal tissue, which later becomes localized at certain parts, and thus the nodule acquires a very uneven surface and shape see (Pl. XIII, Fig. 1). The vascular supply forms one broad zone across the base of the nodule, which subsequently branches and produces a varying number of strands. The bacteroidal tissue becomes separated into a number of distinct areas with a varying amount of sterile tissue between them (see Text-fig. 2). The nodule is protected by a relatively large development from a well-defined phellogen of regular layers of cells, of which large areas are repeatedly split off and renewed as the nodule grows (see



TEXT-FIG. 2. *a*. Longitudinal section of nodule and root of Lupin. *b*. Longitudinal section of an older nodule. × 20.

Pl. XIII, Fig. 8). Infection threads are rarely produced; in fact, Beijerinck says none are formed at all in Lupin. The nuclei with prominent nucleoli remain conspicuous for a long time in the bacteroidal cells.

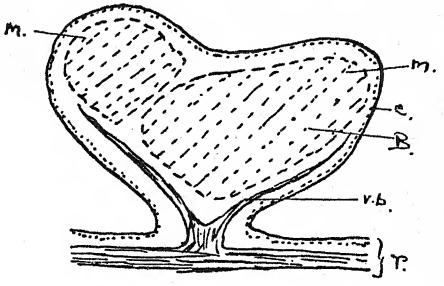
Plants with this type of nodule are further linked together by being woody and having a copious development of periderm; many are shrubs, e.g. *Genista*, *Ulex*, *Amorpha*, and Laburnum is a tree. They can all live on poor dry sandy soil, and some where there is little or no calcium carbonate, e.g. Lupin. They are further characterized anatomically by the presence of schizogenous secretory cavities with tanniniferous contents which are colourless in the living plant, but become brown by the action of oxidizing agents and drying. Curious four-sided prismatic structures also occur in the parenchymatous tissue, which are crystals of calcium oxalate and have been called styloids. The medullary rays traverse the secondary xylem obliquely, forming a kind of net-like pattern and producing knot-like swelling in the neighbourhood of the xylem parenchyma. Tannin sacs and styloids also occur in the nodules, and the parenchyma is more

active than in nodules of other genera, since its development causes the separation of the bacteroidal tissue into several zones.

This group may be subdivided thus:

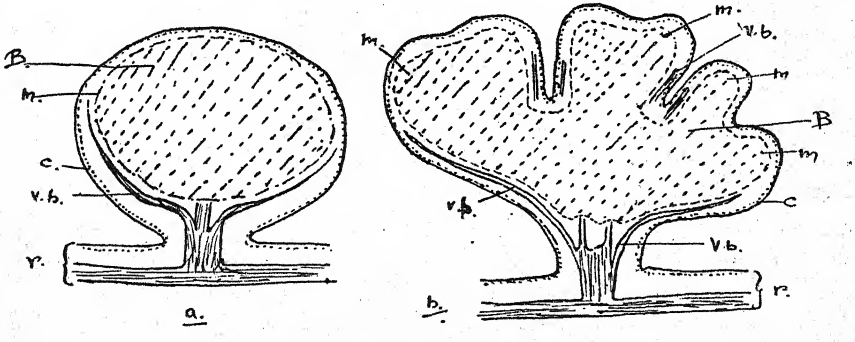
(*a*) In *Lupinus*, *Ornithopus*, *Cytisus*, and *Desmodium* the primary vascular strand is tetrarch and divides into four, correlated with which four growing points are differentiated. The bacteroidal zones become separated by a broad band of parenchyma, in the centre of which a vascular strand appears (see Text-fig. 2).

(*b*) In *Genista*, *Ulex*, *Spartium*, and *Amorpha* the primary vascular strand at the base is diarch and divides into two strands which traverse the nodule some distance before branching again. The bacteroidal zones are separated by a narrow band of parenchymatous cells. In the old nodule of *Ulex* an apical meristem is differentiated, but more usually there is one to each bacteroidal zone (see Text-fig. 3).



TEXT-FIG. 3. Longitudinal section of nodule and root of *Spartium*. × 20.

II. The Phaseoleae and Trifoleae type of nodule are very similar to those above (Pl. XIII, Fig. 2), but the bacteroidal tissue always remains an undivided central zone. The growing point at an early stage frequently becomes localized apically; consequently they elongate, although usually



TEXT-FIG. 4. *a*. Longitudinal section of nodule and root of *Phaseolus*. × 10. *b*. Longitudinal section of old nodule and root of *Lotus corniculatus*. × 15.

remaining very narrow, e. g. *Trifolium*, and frequently the apical meristem branches so that a repeatedly branched nodule may result, e. g. *Lotus corniculatus* (see Text-fig. 4 and Pl. XIII, Fig. 3).

There is greater development of infection threads than in the previous groups. Dawson described them as being present only in the young

nodules of *Phaseolus* and *Coronilla*; Peirce has described them in *Trifolium*; the writer has frequently observed them in young nodules. The nucleus in the young bacteroidal cells is well defined, with a prominent nucleolus, but as the cell increases in size it becomes associated with a large central vacuole. In *Lotus corniculatus* frequently two nucleoli are present, and in *Coronilla* the nucleus becomes amoeboid and subsequently divides so that many cells are multinucleate.

Many of these plants have a very wide distribution and show an immense adaptation to their environment, e.g. *Trifolium* and *Lotus*. They live principally on poor dry sandy soil like the plants of the Genisteae type, and like them produce characteristic tannin sacs, styloids, and oblique medullary rays. Styloids are especially prominent in the outer tissue of the nodule of *Phaseolus* (see Pl. XIII, Fig. 9). These nodules all have one vascular strand at the base and may be grouped thus:

(*a*) *Phaseolus*, *Coronilla*, and others where the vascular strand divides at the base below the bacteroidal tissue into four strands, which supply the nodule for some time before any further branching occurs.

(*b*) *Ononis*, *Anthyllis*, and others in which the vascular strand divides at the base into two.

(*c*) *Trifolium*, *Lotus*, and others where the single strand passes from the base obliquely to one side of the nodule and remains undivided for some time; later it branches, but at some distance from the base.

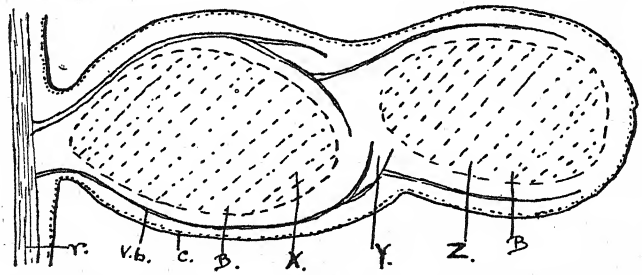
III. In the Viceae type the nodules have an elongated form with a well-defined apical meristem and a basal intercalary zone which produces a small amount of tissue. The nodule frequently branches and may form very large clusters, e.g. *Vicia Faba* and *Stizolobium*; but there is one continuous bacteroidal zone, the apical portions of which are traversed by innumerable infection threads. In young nodules these threads are distributed throughout the whole tissue, but later they are confined to the areas where new cells are continually being formed and infected with *Bacillus radicicola*. The nuclei, which are at first large and prominent, lose their spherical outline, become elongated and irregular, and eventually separate into two or more irregular granular bodies associated with a large vacuole, and later become further dispersed (see Pl. XIII, Fig. 10). Two vascular strands are produced at a very early stage of the development of the nodule on opposite sides, each of which has a separate attachment to the root-stele (see Pl. XIII, Fig. 7).

In this group are a large number of plants of considerable agricultural value which require a moderately good soil with sufficient clay to keep it moist and calcium carbonate to prevent acidity. The genera *Vicia*, *Pisum*, *Lathyrus*, *Galega*, *Stizolobium*, and *Colutea* have been examined and found to have nodules of this type.

IV. The nodules of the above groups all occur on annual plants or are

produced each spring on the new roots and develop through the growing season until the autumn, when they decay in the soil. Beijerinck drew attention to the perennial nature of the nodules of *Robinia*. The author has examined nodules of *Robinia Pseudacacia*, *Sophora*, and some species of *Acacia*, all of which last for more than one growing period. These plants are trees or woody undershrubs which are indigenous in the west temperate and sub-tropical regions. The climatic conditions in these parts would naturally enable the bacteria to retain their activity for a much longer period than in colder regions, and in the nodules of these plants there is a copious development of protective tissue from the phellogen, consisting of thick-walled cells which are very dark in colour owing to the formation of tannin in *Robinia* and *Acacia*, but in *Sophora* they remain much paler, no tannin being present.

These three genera represent three quite different types of Leguminosae, *Acacia* being a very large genus in the Mimosoideae, while



TEXT-FIG. 5. Longitudinal section of nodule and root of *Sophora*. × 15. *x* is the first bacteroidal zone produced; *z*, that produced during the second growing period; *y*, the area between them, in which the vascular strands anastomose.

Sophora and *Robinia* are both members of the Papilionaceae; but *Sophora* represents the unusual type in which the stamens are free, and *Robinia* is one of the largest trees in the order. They all have characteristic pinnate leaves with a large number of pinnules adapted to a greater or less degree for movement in response to external stimuli.

The nodules all develop two vascular strands which have a separate attachment to the root-stele, and a well-developed bundle sheath is present. In *Acacia* the meristem extends all round the bacteroidal tissue and its activity is renewed from time to time. But the greatest growth always takes place so that the nodule becomes bean-shaped (see Pl. XIII, Fig. 4). In *Sophora* and *Robinia* the growing point is apical and the nodule becomes elongated and transversely indented. The indentations occur between two periods of growth, and consist of cortical cells which in *Sophora* are transversed by the vascular strands which anastomose in this area (see Text-fig. 5).

Infection threads have been observed in connexion with the migration of the bacteria into the newer bacteroidal cells. These cells elongate in the

direction of the length of the nodule, become multinucleate, and later the nuclei disappear. Many empty nodules were also found on the roots, indicating that they only last for a limited period which does not correspond with the life of the root as in non-leguminous nodules. This type may be called the Mimosoideae type.

The amount of nitrogen which is fixed by *Bacillus radicicola* has been thought to be connected with the quantity of slime which is produced under the given circumstances. When the development of slime is large the bacteria are held in it and form what is called a zoogleal thread. It is in this form that they are very active in entering the root-hairs and passing from cell to cell of the nodule. In the Viceae type the zooglea persists much longer than in the other groups in the cell of the nodule itself. Eventually the slime is absorbed and the bacteria live freely in the cell, then in many cases they no longer remain rod-shaped but assume a characteristic form, e. g. in *Vicia Faba* they become V and Y shape, in *Lotus corniculatus* spherical. These have been called bacteroids. Buchanan has described a large number of these forms both in nodules and artificial cultures and ascertained that their production and formation depend very largely on their nutrition. Many have believed them to be absorbed by the plant, but inside them are small denser spherical bodies, the presence of which renders the organism much more resistant to its environment than it was before, consequently they are more and more abundant as the nodule grows older and the season advances.

The nuclear changes which have been observed above are associated with the presence of the zooglea. It is in those cells in which the zooglea persists and envelops the nuclei that the latter become amoeboid and divide amitotically before the cell becomes a permanent cell with very little protoplasmic content at all. This has also been recorded in non-leguminous nodules; e. g. in the Podocarpineae and Elaeagnaceae the nuclei behave as described above for the Viceae type, but have not been observed to do so in *Alnus*, although it is so much like *Elaeagnus* in other respects. The presence of the zooglea apparently has a stimulating effect upon the nucleus.

In artificial cultivation it has been observed that the amount of slime produced is largely influenced by the medium used, e. g. the kind of carbohydrate supplied. Bacteria obtained from nodules of different plants also behave rather differently towards the carbohydrate supplied, some responding more readily to maltose, others to sucrose, and so on. They all can utilize a very large number of carbohydrates, but become particularly adapted to one form, probably by growing in the root of a plant which contains that one. Buchanan found the production of gum or slime in some cases was favoured by the presence of sodium succinate, ammonium citrate, glycerine, glucosides, and inhibited by asparagin, whilst in other cases

it is entirely the reverse. It has, however, been demonstrated generally that the addition of a trace of nitrogenous material behaves as a stimulant for the rapid production of slime, and Mockeridge found the presence of an organic substance, e.g. a humate, had a very marked effect. The acidity or alkalinity of the medium also plays a prominent part. Moore found that the presence of from 0·33 to 1 per cent. of potassium and sodium salts was often sufficient to inhibit the formation of nodules, whilst the same amount of calcium and magnesium salts favoured their production.

It therefore seems probable that the nature of the cell-sap of the root will determine not only whether the bacteria shall enter and multiply, but also the production of infection threads, which can only be produced in the presence and absence of certain definite substances in the medium. This in the plant is the cell, and the particular nature of the cell-sap it contains will be influenced by that of the soil in which the plant is growing. It is consequently of interest that they are absent from the Genisteae type and only slightly developed in the Phaseoleae and Trifoleae type, which flourish in poor sandy soil, but play an important part in the Viceae and Mimosoideae types, which normally inhabit a richer damper soil. In the Genisteae type also, where they are absent, the plants grow where there is a deficiency of calcium carbonate. There thus seems to be a variation in the morphology of the nodule resulting from infection produced primarily by the possible response of the bacteria to the cell-sap of the host, and the presence of the bacteria induces growth on the part of the host which will naturally be influenced by the anatomical peculiarities of that plant.

Summary.

1. Plants which produce root-tubercles when infected with the nitrogen-fixing organism *Bacillus radicicola* are of two kinds—legumes and non-legumes.

2. The root-tubercles of non-legumes are modified lateral roots, but those of legumes are exogenous in origin.

3. In the leguminous nodule the bacteroidal tissue is central and the vascular system consists of a number of peripheral strands.

4. *Bacillus radicicola* is connected with the assimilation of nitrogen from the atmosphere, and although it is polymorphic, cross-inoculation occurs.

5. The leguminous nodules can be placed in four groups:

I. The Genisteae type, in which the meristem is spherical, the vascular supply forms one broad zone across the base, and the bacteroidal tissue becomes divided into several parts.

(*a*) In *Lupinus* the primary vascular strand is tetrarch and the bacteroidal zones are separated by vascular as well as parenchymatous tissue.

(*b*) In *Genista* the primary vascular strand is diarch and the tissue between the bacteroidal zones is all parenchyma.

II. The Phaseoleae and Trifoleae type, in which the bacteroidal tissue always remains an undivided central zone, the growing point becomes localized apically, and zoogleal threads are prominent in the young nodules.

(*a*) In *Phaseolus* the basal vascular strand divides into four.

(*b*) In *Ononis* the basal vascular strand divides into two.

(*c*) In *Trifolium* the single strand remains undivided for some time.

III. The Viceae type, in which there is a well-defined apical meristem with a basal intercalary zone. Zoogleal infection threads are very prominent. Two vascular strands have a separate attachment to the root-stele.

IV. The Mimosoideae type, in which the nodules persist for more than one year.

6. When the zooglea persists and envelops the nuclei, the latter become vacuolate, then amoeboid, and later divide amitotically.

7. After the disappearance of the infection threads the bacteria assume a variety of forms known as bacteroids.

8. The production of slime is connected with the amount of nitrogen fixed, and is influenced by the medium in which the bacteria are living.

9. The nature of the cell-sap has a marked influence on the capabilities of the bacteria.

10. The form of the nodule depends primarily on the nature of the environment of the host, which influences the cell-sap and consequently the behaviour of the bacteria after they have entered, and secondarily on the anatomical peculiarities of the particular plant.

Bibliography.

Beijerinck, M. W.: Die Bakterien der Papilionaceenknöllchen. Bot. Zeit., vol. xlvi, 1888.

Bottomley, W. B.: The Cross-inoculation of the Nodule-forming Bacteria from Leguminous and Non-leguminous Plants. Report Brit. Assoc., 1907.

———: The Root-nodules of *Myrica Gale*. Ann. Bot., vol. xxvi, 1912.

Brunchorst, J.: Ueber die Knöllchen an den Leguminosenwurzeln. Ber. d. Deutsch. Bot. Ges., vol. iii, 1885.

Buchanan, R. E.: The Gum produced by *Bacillus radicicola*. Centralbl. f. Bakt., Abt. ii, Bd. xxii, 1909.

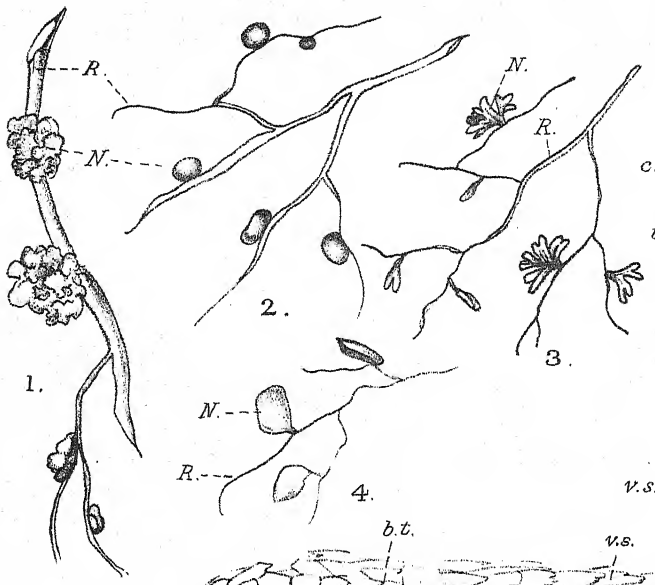
DAWSON, M.: Nitragin and the Nodules of Leguminous Plants. Phil. Trans., B., vol. cxcii, 1900.
FRANK, A. B.: Die Pilzsymbiose der Leguminosen. Landwirthschaft. Jahrb. xxi, Berlin, 1892.
HELLRIEGEL, H., and WILFARTH, H.: Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. Zeit. des Vereins für Rübenzucker-Industrie, 1888.
MAZÉ: Les microbes des nodosités des Légumineuses. Ann. Inst. Pasteur, xii, 1898.
MOCKERIDGE, F. A.: Some Effects of Organic Growth-promoting Substances (Auximones) on the Soil Organisms concerned in the Nitrogen Cycle. Proc. Roy. Soc., B., vol. lxxxix, 1917.
MOORE, G. F.: Bulletin 71 of Bureau of Plant Industry, Washington, Jan., 1905.
NOBBE and HILTNER: Ueber die Anpassungsfähigkeit der Knöllchenbakterien ungleichen Ursprungs an verschiedene Leguminosengattungen. Landwirtschaftliche Versuchsstationen, vol. xlvii, 1896.
PEIRCE, J. G.: The Root-tubercles of Bur Clover and of some other Leguminous Plants. Proc. of the California Acad. of Sciences, 1902.
PRAZMOWSKI, A.: Die Wurzelknöllchen der Erbse. Landw. Versuchsstationen, 1890 and 1891.
SOLEREDER: Systematic Anatomy of Dicotyledons, 1908.
SPRATT, E. R.: The Morphology of the Root-tubercles of *Alnus* and *Elaeagnus* and the Polymorphism of the Organism causing their Formation. Ann. Bot., vol. xxvi, 1912.
————: The Formation and Physiological Significance of the Root-nodules in the Podocarpaceae. Ann. Bot., vol. xxvi, 1912.
————: The Root-nodules of the Cycadaceae. Ann. Bot., vol. xxix, 1915.
WARD, H. M.: On the Tubercular Swellings on the Roots of *Vicia Faba*. Phil. Trans. Roy. Soc., Lond., 1887, vol. clxxviii.
WILLIS: Flowering Plants and Ferns, 1908.
WINOGRADSKY: Sur l'assimilation de l'azote gazeux de l'atmosphère par les microbes. Compt. rendus, Paris, 1893.

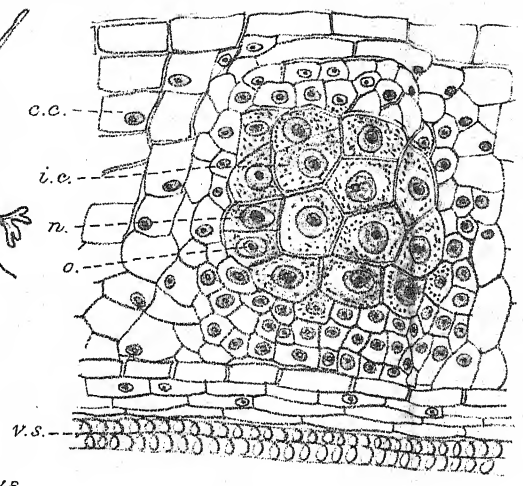
DESCRIPTION OF PLATE XIII.

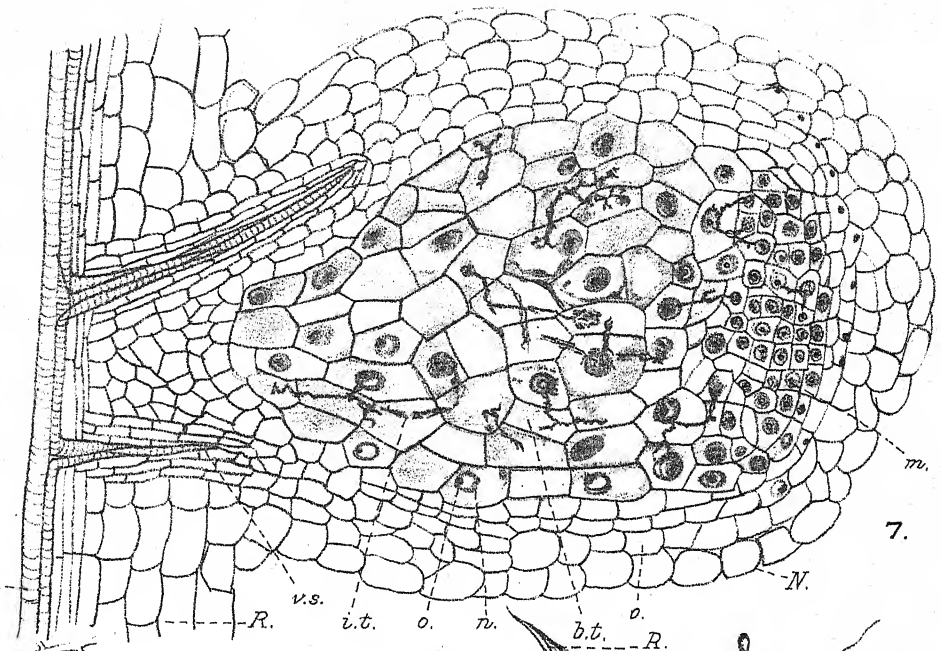
Illustrating Dr. Ethel R. Spratt's paper on Root-nodules of the Leguminosae.

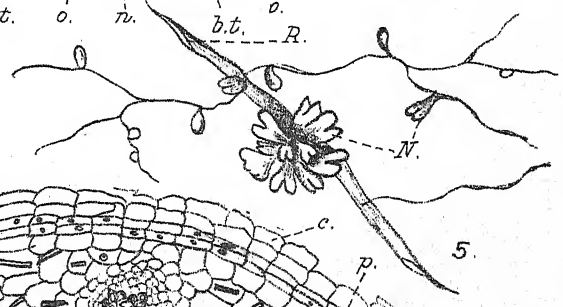
R. = root; *v.s.* = vascular strand; *b.t.* = bacteroidal tissue; *i.t.* = infection thread; *m.* = meristem; *S.t.* = root-stele; *c.* = outer protective layers; *n.* = nucleus; *o.* = nucleolus; *N.* = nodule.

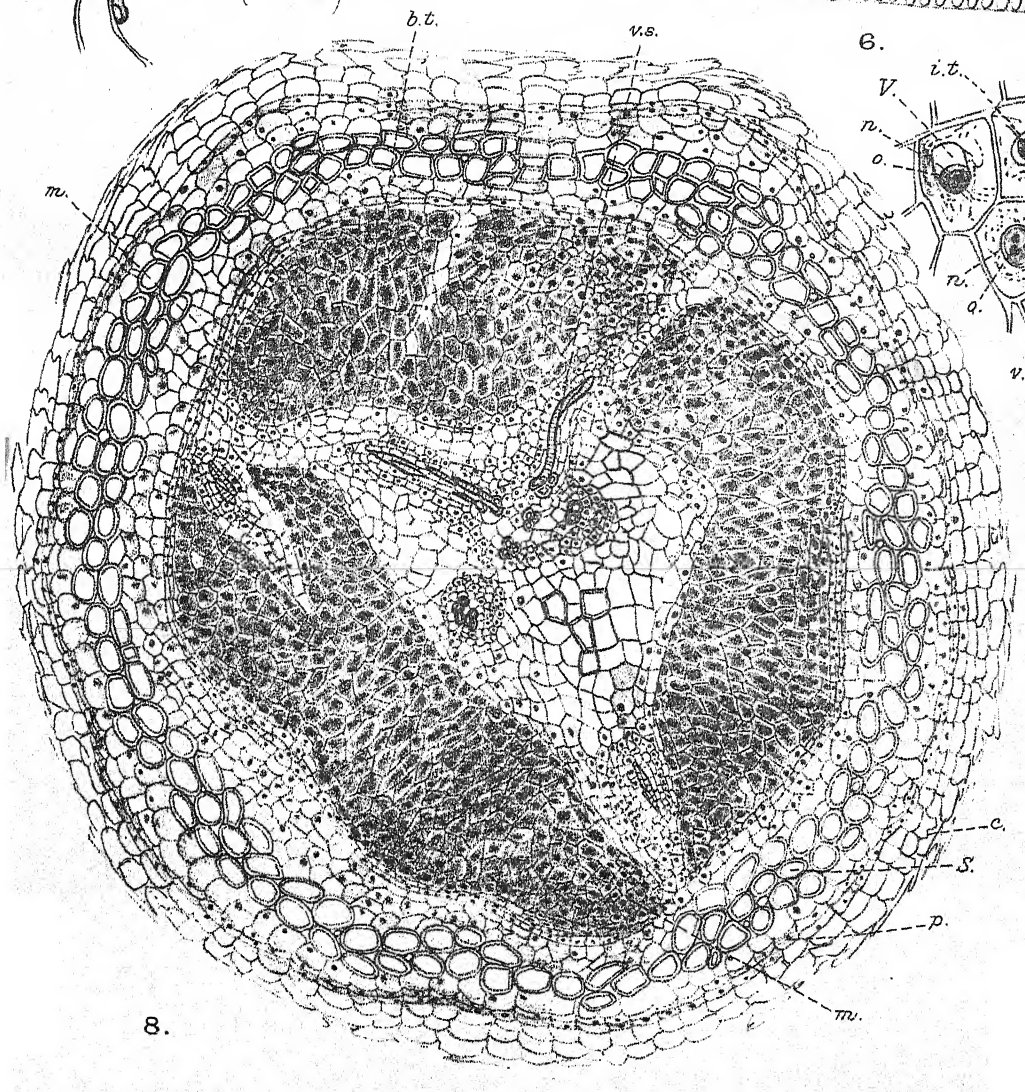
Fig. 1. Roots of *Lupinus* with nodules. Nat. size.
Fig. 2. Roots of *Phaseolus* with nodules. Nat. size.
Fig. 3. Roots of *Lotus corniculatus* with nodules. Nat. size.
Fig. 4. Roots of *Acacia* with nodules. Nat. size.
Fig. 5. Roots of *Vicia Faba* with nodules. Nat. size.
Fig. 6. Part of longitudinal section of a root of *Vicia Faba*, showing the cortical cells (*c.c.*), some of which, *i.c.*, have become infected with *Bacillus radicicola*. × 325.
Fig. 7. Longitudinal section of nodule of *Vicia Faba*. × 325.
Fig. 8. Transverse section of nodule of *Lupinus*, showing three zones of bacteroidal tissue. *p.* = phellogen; *s.* = thick-walled cells. × 70.
Fig. 9. Transverse section of nodule of *Phaseolus*. *p.* = phellogen; *y.* = styloids. × 70.
Fig. 10. Bacteroidal cells of *Lathyrus odoratus*, showing nuclear changes. *v.* = vacuole. × 325.

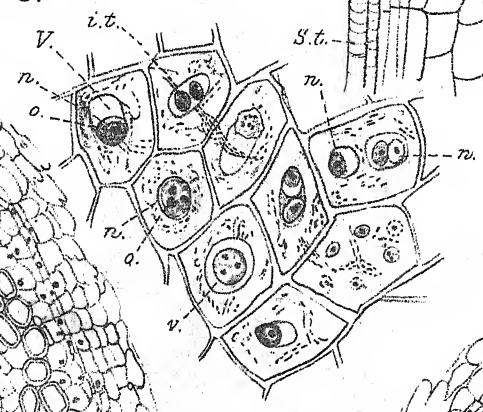


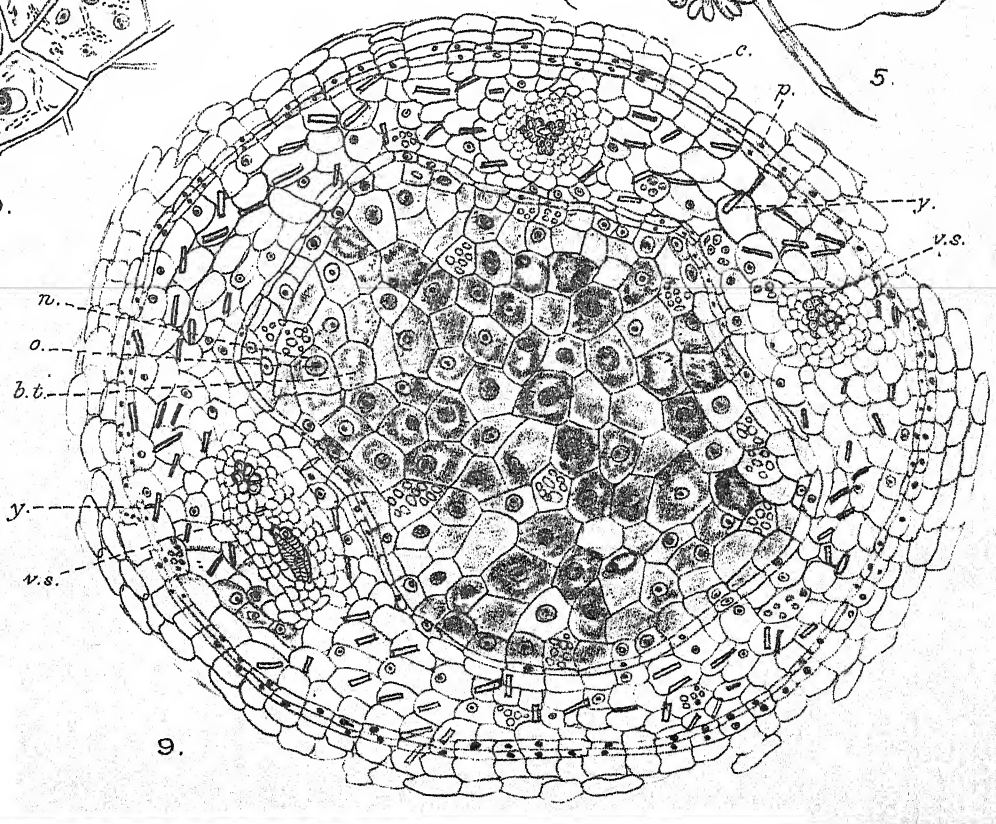












Huth, Lith.r London.

E. R. SPRATT.

Induced Changes in Reserve Materials in Evergreen Herbaceous Leaves.

BY

GWYNETHE M. TUTTLE, M.Sc.,

Botanical Department, University of Alberta.

With seven Figures in the Text.

EVERGREEN trees are generally divided into two classes: (*a*) 'starch' trees, whose reserve food is stored in the form of starch, and (*b*) 'fat' trees, in which the starch is converted into oil during the autumn. According to Warming (1), the presence of oils and fats in the latter may enable the plant to withstand lower temperatures, 'in that fatty oil in the form of an emulsion prevents the sub-cooling of the plant tissue and increases the power of resistance to frost'. Hence we find this class of trees largely distributed throughout the colder regions, while the 'starch' trees are found in localities of higher winter temperature.

Much attention has been given to this phenomenon in trees, but no information is available in connexion with herbaceous plants. Since many evergreen herbaceous plants grow in the shade of the 'fat' trees subjected to the same extremes of cold, it would seem possible that they should exhibit similar features. It was with a view to ascertaining a few facts in this connexion that the present work was undertaken. My attention was called to the need for investigation in this direction by Dr. F. J. Lewis, to whom I am greatly indebted for helpful criticism and suggestions given throughout the progress of the work.

The only recent investigation bearing on this subject is that of Küchi Miyake (2) at Tokyo. He confined his attention solely to the calculation of the winter starch content of a number of trees and herbaceous plants from different parts of Japan. The conclusion is reached that the starch content of trees in winter is very small compared with that during the summer, but that the content varied with trees of different species and also with the region from which the material came—that from Northern Japan,

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

where lower winter temperatures occurred, showed less starch than that from the southern districts. Küchi Miyake simply recorded the starch content as he found it. He did not try to account for its variation. It would have been interesting to know whether part of this decrease in starch was due to the formation of fats and oils at the beginning of winter, but no mention is made of investigation in this direction.

The present investigation was undertaken, therefore, not so much with a view to ascertaining the food reserve in a large number of plants as that of a single one, *Linnaea borealis*, L. var. *Americana*, Forbes, and to investigate the changes which these reserves undergo when subjected to different temperature conditions.

In view of the fact that temperature is vitally connected with the problem, a brief statement may be given of the meteorological conditions of the district. Early frosts are characteristic of Western Canada, occurring usually from the 6th to the 10th of September—frequently followed by warmer periods. The precipitation during September and October is usually high, but varies considerably with the different years. The winter seasons are quite variable, but the extreme temperatures remain the same from year to year, ranging from about −40° F. (−40° C.) to an occasional 50° F. (10° C.). The humidity of the air is moderately low and high winds are rare. The depth of snow averages from one to two feet on the level.

The winter 1916–17, however, has been unusual. Cold periods of five to ten days in which the thermometer ranged from 0° F. (− 17° C.) to − 40° F. (−40° C.) have alternated quite regularly with slightly longer periods of rising temperature, reaching on one occasion 47° F. (8° C.). As will be mentioned later, these fluctuations have been useful during the progress of the investigation, especially in connexion with reconversion.

I. Preliminary Tests of Reserves.

The work was commenced late in October, just about the time of leaf-fall of deciduous trees. A number of leaves were examined then for their starch and oil content. A solution of iodine and another of chloral hydrate and iodine were used in the tests for starch, the former in the preliminary tests and the latter in the material during conversion, as the chloral hydrate swelled the starch grains and also decolorized the sections, thus making the reaction much clearer. For fats and oils, sections of the material were tested with a 1 per cent. solution of osmic acid and also with neutral red solution, both tests yielding similar results.

Table I shows the starch and oil content of the majority of local plants which still retained their leaves.

TABLE I.

Fall Starch and Oil Content of Leaves.

Date.	*Material.*	*Starch.*	*Oils and Fats.*
Oct. 23	*Picea canadensis*, Mill (B. S. P.)	Very little	Large amount
,, 23	*Pyrola rotundifolia*, L.	None	,, ,,
,, 23	*Linnaea borealis*, L. var. *Americana* (Forbes), Redner	Very little	,, ,,
,, 24	*Cornus canadensis*, L.	None	,, ,,
,, 26	*Hypnum* (sp.)	Good reaction	None
,, 26	*Marchantia* (sp.)	Very little	Large amount
,, 26	*Mitella nuda*, L.	None	,, ,,
,, 28	*Pinus Banksiana* (Lamb)	Very little	,, ,,

It will be seen from the table that all the evergreens were practically destarchified, and on the other hand they contained a large amount of oil.

II. CONVERSION OF OILS AND FATS TO STARCH.

A series of experiments was then started to induce the formation of starch in the destarched plants. The following plants were brought indoors and subjected to higher temperatures: *Linnaea*, *Pyrola*, and *Picea*. The first two plants were potted, but in the case of *Picea* shoots were kept in water. The experiments were kept in a dark room at a temperature of 68° F. (20° C.), and in each case a control was placed outside in the dark at the lower temperature.

The material of *Linnaea* showed starch appearing as early as the second day, while *Pyrola* and *Picea* showed no increase, although allowed to remain at the higher temperature for five weeks. This points to the fact that different species vary in their response to the change in temperature.

As *Linnaea* responded most readily to experiment, the following observations were all made from that plant. They are based on material collected and potted at intervals of five or six days during November and December, 1916, and January, 1917. In each case the result was based on a test from six to twelve leaves.

TABLE II.

Starch in Material exposed to Higher Temperature.

Date.		*Condition when potted.*	*1st day.*	*2nd day.*	*3rd day.*	*4th day.*	*1 week.*
Nov. 8	experiment	No starch	No test	No test	No test	No test	Good reaction
	control	,,	,,	,,	,,	,,	No starch
,, 14	experiment	,,	,,	,,	Good reaction	,,	Strong reaction
	control	,,	,,	,,	No starch	,,	No starch
,, 16	experiment	,,	,,	,,	No test	Good reaction	Strong reaction
	control	,,	,,	,,	,,	No starch	No starch
,, 23	experiment	,,	,,	,,	,,	Good reaction	Strong reaction
	control	,,	,,	,,	,,	No starch	No starch
Dec. 4	experiment	,,	No starch	Slight reaction	,,	Good reaction	Strong reaction
	control	,,	,,	No reaction	,,	No starch	No starch
,, 18	experiment	,,	,,	Slight reaction	,,	Good reaction	Strong reaction
	control	,,	,,	No reaction	,,	No starch	No starch
,, 30	experiment	,,	No test	Slight reaction	,,	Good reaction	Strong reaction
	control	,,	,,	No reaction	,,	No reaction	No reaction

Material which has been inside for two days shows a starch reaction in all parts of the leaf. At the end of four days the cells are quite crowded with starch, but the amount increases until the seventh or eighth day (Fig. 1), after which time the starch content seems to remain constant.

The starch in all cells is present in a very finely divided state and the individual grains exhibit Brownian movement to a marked degree.

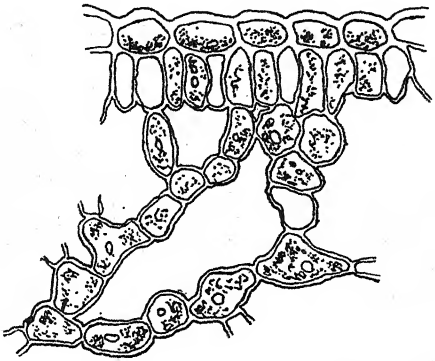


FIG. 1. Cross-section of *Linnaea* leaf, showing starch in material exposed to higher temperature for one week. Section treated with chloral hydrate and iodine.

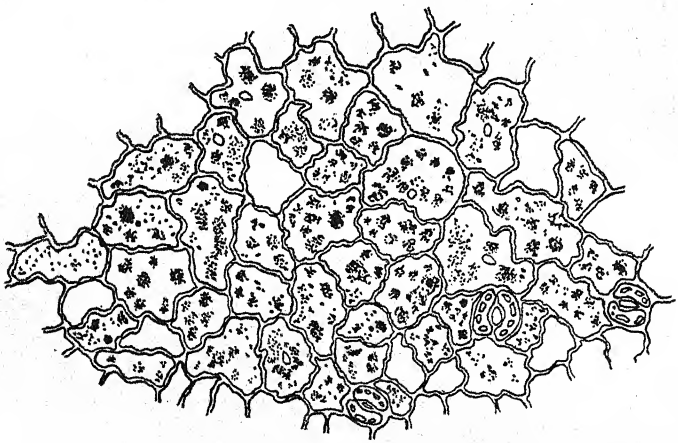


FIG. 2. Horizontal section of *Linnaea* leaf, showing starch in epidermal cells. The material had been exposed to higher temperature for one week. Section treated with chloral hydrate and iodine.

It is interesting to note the large amount of starch present in the epidermal cells of material undergoing conversion (Fig. 2), as none is found there during the summer when the starch is being formed as a product of photosynthesis—this apparently suggesting that the starch has arisen by conversion of oils and fats which are abundant in the epidermis during the

winter (Fig. 3). It has been observed that the starch grains are grouped as though each group had arisen from a single oil globule.

No regular variation in the conversion has been determined. Entire plants have been tested, and it has been shown that they do not possess 'fat' leaves and 'starch' leaves—all leaves tend to form starch when exposed to higher temperature. Fig. 4 shows the test of three plants from material which had been exposed to higher temperature for four days.

In this test, plants I and III gave a strong reaction in all of the leaves, but very little reaction was obtained in any leaves from plant II.

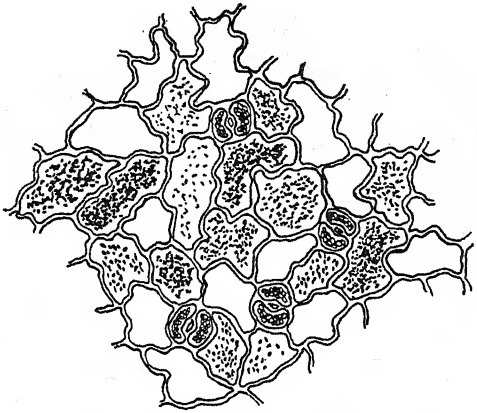


FIG. 3. Horizontal section of *Linnaea* leaf, showing oils and fats in epidermal cells. Material fresh from outside before exposure to higher temperature. Section treated with 1 per cent. osmic acid.

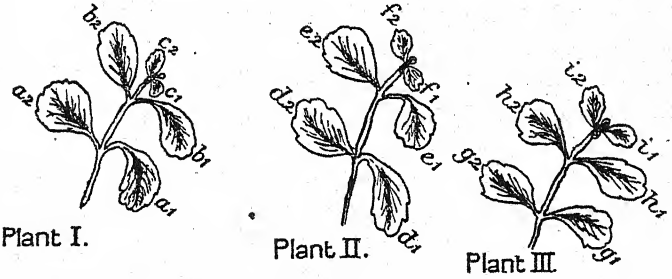


FIG. 4. *Linnaea* material exposed to higher temperature for four days, sections of which were treated with chloral hydrate and iodine.

Strong starch reaction in sections from a_1–a_2–b_1–b_2–c_2, g_1–g_2–h_1–h_2–i_1–i_2.
Slight starch reaction in sections from e_1–f_2.
No " " " " " e_2–d_1–d_2.

This may be due to the fact that its leaves were not so vigorous as those of plants I and III. Hence any variation seems to be due to some plants being more vigorous than others, and also to the age of the leaves, as it has been observed that younger leaves carry on the conversion much more rapidly than do the older ones.

The increase in the starch content of these leaves is much more evident than the decrease in the oils and fats which one would suppose must be taking place, as that seems the only source from which the starch can be derived, for the factor of photosynthesis has been eliminated by placing the material in darkness. All the leaves showing a large amount of starch gave a strong reaction with the test for oils and fats. In horizontal sections passing through the epidermis there seems to be quite as much oil as starch present. The palisade tissue shows no appreciable decrease. It is only in the cells of the mesophyll that an actual difference has been recognized. Here the decrease is quite evident, as will be seen by comparing Figs. 5 and 6.

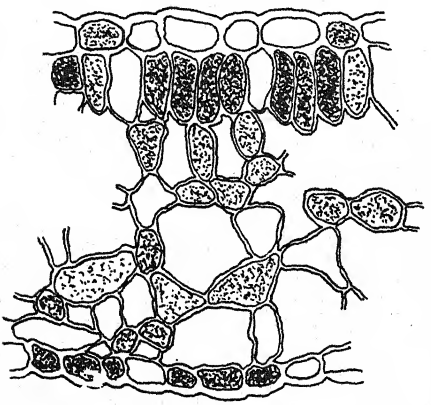


FIG. 5. Cross-section of *Linnaea* leaf, showing fats and oils before exposure to higher temperature. Section treated with 1 per cent. osmic acid.

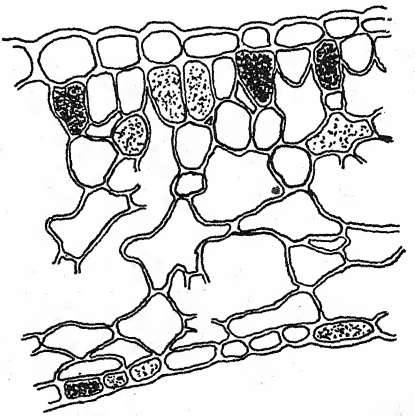


FIG. 6. Cross-section of *Linnaea* leaf, showing decreased fat and oil content. Material exposed to higher temperature for one week. Section treated with 1 per cent. osmic acid.

III. RECONVERSION OF STARCH INTO FATS AND OILS.

Having shown that starch appears in those leaves subjected to a higher temperature, it was then attempted to cause a reconversion of the starch to oils and fats by lowering the temperature. Material of *Linnaea* which had been kept at 68° F. (20° C.) for a week, and which showed as a result a high starch content, was placed outside in darkness on two different dates—November 25 and December 13. As will be seen from Tables III and IV, the temperature conditions were very different during the two experiments.

TABLE III.

Material containing Starch exposed to moderately low Temperature.

Date.	*Av. Temperature.*	*Experiment.*	*Control.*
Nov. 25	25° F. (− 3° C.)	Strong starch reaction	Strong starch reaction
„ 30	36° F. (2° C.)	Decreased starch content, four leaves out of six destarchified	„ „
Dec. 4	28° F. (− 2° C.)	All material destarchified	„ „

TABLE IV.

Material containing Starch exposed to extremely low Temperature.

Date.	*Av. Temperature.*	*Experiment.*	*Control.*
Dec. 13	4° F. (− 15° C.)	Strong starch reaction	Strong starch reaction
„ 20	− 16° F. (− 26° C.)	No decrease in starch, cells strongly plasmolysed	„ „
„ 23	− 19° F. (− 28° C.)	„ „	„ „

From Tables III and IV it is seen that material containing a large amount of starch undergoes reconversion, in which the starch is changed back into oils and fats if subjected to moderately low temperature. If, however, the material, full of starch and hence in a summer condition, is subjected to extremely low temperatures no reconversion takes place and the material is killed. The strong plasmolysis of the cell contents exhibited in this material is shown in Fig. 7.

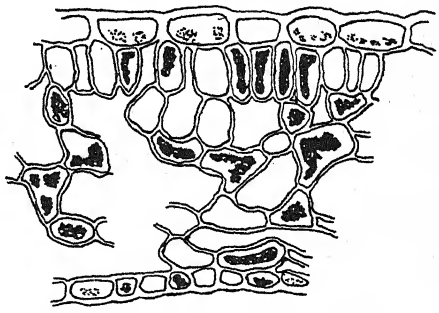


FIG. 7. Cross-section of *Linnaea* leaf. Material outside for reconversion during extremely low temperature. Section treated with iodine solution. Starch grains visible in epidermic cells. Owing to the high starch content, the plasmolysed cell contents were stained deep blue.

IV. CAUSE OF CONVERSION.

The conversion of fats to starch is quite impossible as an unaided chemical change, and hence the action of an enzyme is suggested. Tests were made for the presence of an enzyme both in material fresh from outside and in that which had been exposed to higher temperature for some time.

The test in this case was an alcoholic solution of guaiacum resin, which, with the addition of hydrogen peroxide, gives a blue reaction in the presence of any enzyme. In enzyme tests made December 7 and 18 in fresh material no reaction was obtained. Material, however, which had been exposed to higher temperature for several days gave a strong reaction when tested.

About a month later (Jan. 11–16) material in the same condition was tested which yielded rather different results. In the interval, however, the weather had moderated and some very high temperatures had been recorded. All *Linnaea* leaves, both from outside and inside, then responded very strongly to the enzyme test. There was an inclination at first to doubt the test, but when leaves of Begonia and Geranium from a greenhouse gave negative results it was concluded that the change in temperature must have affected the outside material. Still the change had not been great enough to

cause the formation of starch, for the outside material gave no reaction to the iodine test. The effect of changing temperature was watched, and it was found that the enzyme response diminished after a fortnight of lower temperature, although at that time a slight reaction was given despite the fact that the material was gathered when the temperature was lower than 0° F. (– 17° C.).

The important point, however, was that the material undergoing conversion gave a strong enzyme reaction. The next step was to determine whether or not this was due to the fat-splitting enzyme lipase. The test applied was that used by G. J. Fowler (3) and Reynolds Green (4) in work with germinating *Ricinus* seeds. Here the solution which is to be tested for the presence of the enzyme is allowed to act on an oil emulsion causing (if lipase be present) the hydrolysis of the oil and the formation of a fatty acid.

An extract of the leaves was made by grinding them in a mortar with a solution containing 5 per cent. sodium chloride and 0·2 per cent. thymol as an antiseptic. This was allowed to stand for twenty-four hours and then filtered. The filtrate was treated as follows: an emulsion of olive oil with a little gum arabic was made, and to this was added a small amount of the extract, phenolphthalein being used as an indicator. As the leaf extract and oil emulsion were both slightly acid, they were carefully neutralized with a few drops of very dilute caustic potash. From two to five experiment tubes were made up in this manner. A control was made up in a similar manner, except that the extract was boiled for two or three minutes before it was added to the emulsion. Both experiments and control were placed in an oven at 95° F. (35° C.). Table V gives the results of several tests for lipase in extracts from material of different conditions. The test made on January 9 shows that fresh material from outside without exposure to higher temperature does not respond to the test for lipase.

TABLE V.

Action of Lipase.

Date.	*Exposure to Higher Temperature.*	*Experiment.*	*Boiled Control.*	*Indicator.*
Dec. 4	4 days	Acid reaction after 4 hours	No reaction	Litmus
,, 8	4 ,,	Acid reaction after 4 hours	,,	Phenolphthalein
Jan. 2	2 ,,	Acid reaction after 2 hours	,,	,,
,, 9	None (material tested when brought in)	No reaction	,,	,,
,, 16	2 days	Acid reaction after 2 hours	Slightly acid [1]	,,
Feb. 8	2 ,,	Acid reaction after 4 hours	No reaction	,,

[1] Due to insufficient boiling.

It has been recorded earlier in the paper that material fresh from outside responded to the general enzyme test. Experiment of January 9 in Table V shows that the enzyme in fresh material is not lipase—hence the presence of another enzyme is suggested. Owing to the browning of the leaf extract on standing, oxidases were suggested.

It was determined (3) that guaiacum resin without the hydrogen peroxide was a test for oxidase alone, and so the material was subjected to this test. Sections of the leaves, both fresh and converting, gave only slight reactions with the guiacum resin, but the extract gave a good response. This is due to the fact that, on standing, a substance is formed in the extract which increases the oxidation (5).

In the tests for lipase given above it will be noticed that phenolphthalein was used as an indicator instead of the litmus solution used by Reynolds Green in his experiments with extract from endosperms of *Ricinus*. Litmus was used in the early experiments in this work, but it was found that the plant extract decolorized the litmus directly it was added. This fact was a further proof of the presence of oxidases, as they cause an immediate decolorization of litmus solution. Hence litmus was of no use as an indicator in the presence of oxidases, and so phenolphthalein was substituted, with whose action they do not seem to interfere.

The results of this investigation may be briefly summarized as follows:

1. Most evergreen plants are destarchified in NW. Canada as early as October, and then contain a large amount of oil.

2. Exposure to higher temperature in the case of *Linnaea* induces the formation of starch in darkness.

3. Starch is present in the material after two days and increases in amount until about the eighth day.

4. The starch is in a very finely divided state and the individual grains exhibit Brownian movement.

5. All healthy leaves are capable of carrying on conversion. The plant does not possess 'starch' leaves and 'fat' leaves.

6. The starch disappears when again exposed to moderately low temperature for about eight days, but the leaves are killed if exposed to extremely low temperatures when filled with starch.

7. A decrease in the oil content is evident in leaves which have formed starch by conversion.

8. Enzymes are present in material undergoing conversion.

9. Lipase has been demonstrated in material undergoing conversion.

10. Oxidases are present in the leaf of *Linnaea* even at quite low temperature.

Bibliography.

1. Warming, Eug.: Ecology of Plants. 1909.
2. Küchi Miyake: On the Starch of Evergreen Leaves and its Relation to Photosynthesis during the Winter. Bot. Gaz., xxxiii.
3. Fowler, G. J.: Bacteriological and Enzyme Chemistry. 1911.
4. Reynolds Green, J.: Fermentation. 1901.
5. Wheldale, Muriel: Direct Guaiacum Reaction given by Plant Extracts. Proc. Royal Society, Series B, 84.

On the Law of Age and Area, in Relation to the Extinction of Species.

BY

AGNES ARBER.

DR. J. C. WILLIS, in the well-known series of papers in this and other journals in which he has elaborated his theory of 'Age and Area', has brought forward evidence for its validity which appears to me to be completely convincing. I am, however, unable to accept one subsidiary hypothesis, which Dr. Willis seems to regard as an integral factor in his scheme—the idea that, at least in the case of the Angiosperms, no extinction of species is now proceeding. This opinion he has repeatedly expressed. He wrote, for instance, in 1916[1] that 'There is no evidence whatever that any of the angiospermous species of the Ceylon flora are dying out, and from analogy we may imagine this to be generally true'. He does indeed qualify this in 1918[2] as far as to admit that 'there is a certain amount of geological evidence of former greater spread', but he seems to regard this as an exception of no real importance. Berry,[3] on palaeobotanical grounds, and also Sinnott[4] and Ridley,[5] for more general reasons, have controverted Willis's view that no extinction is taking place among the Flowering Plants, and have expressed the opinion that certain members of this group are dying out at the present day.

When we look at the matter broadly, and consider living things as a whole, it becomes abundantly clear that extinction both of plant and animal species has occurred on a vast scale in the course of bygone geological epochs. I fancy that those palaeontologists who have paid the closest attention to these questions would be the least likely to accept Willis's contention that extinction in the past has been due almost entirely to catastrophes of some kind, or to great climatic changes.[6] Even if we follow Willis in narrowing the issue down to Angiosperms, we must admit that there is no apparent reason for supposing them privileged to escape the universal fate. It might possibly be maintained that at the present day the Angiosperms are a dominant group entirely on the up-grade, in which extinction has not yet begun. But this position is refuted by certain

[1] Willis, J. C. (1916). [2] Willis, J. C. (1918). [3] Berry, E. W. (1917[1]).
[4] Sinnott, E. W. (1917). [5] Ridley, H. N. (1916). [6] Willis, J. C. (1918).

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

palaeobotanical evidence which can scarcely be gainsaid. For the sake of brevity I will cite one instance only. Professor Berry,[1] by bringing together the evidence of Cretaceous and Tertiary fossils, has established that the genus *Nelumbo*, which is now represented by two species only, occurring respectively in Asia and America, had formerly a cosmopolitan range, including Greenland, Europe, and Africa. A number of the species which have been identified in the fossil state are now wholly extinct. A map showing the present and past distribution, which is included in Professor Berry's paper, brings vividly home to the reader the losses which this genus has suffered.

Having become convinced that Willis's position, in regard to the extinction of Angiospermic plants, was untenable, I sought to discover whether the validity of the Law of Age and Area must, in reality, stand or fall with the question of the dying out of species; the conclusion I have reached is that Willis's deductions regarding extinction depend upon a false assumption, and that they may be discarded without in any way affecting the truth of the law.

It will be remembered that Willis bases his hypothesis in the first instance upon a consideration of the *degree of rarity* of the various species constituting the flora of Ceylon.[2] Following Trimen, he classifies the plants into a series of classes grading from 'Very Common' to 'Very Rare', and he uses the ingenious idea of awarding to each species 'marks' for rarity on a numerical scale. He writes, regarding the statistics based upon these classes, 'In what way the figures we have given are to be reconciled with any theory of the dying out of species I fail to understand'.[3] When we scrutinize Willis's degrees of rarity more closely, we see that he uses the words 'common' and 'rare' in a slightly peculiar,[4] though legitimate, sense, which he is careful expressly to define; he lays special stress on the fact that 'my figures . . . refer *to area occupied, not to commonness on the ground*'.[5] This being the case, it seems to me quite impossible to draw any conclusions for or against extinction from the figures in question; *the whole matter hinges upon a confusion of thought between 'common' in the sense of widespread, and 'common' in the sense of numerically abundant.* Willis apparently expects that those who differ from him on the question of extinction ought to be able to 'define a size of area above which species are to be regarded as growing, or below which as dying out'.[6] But what right have we to assume that the mechanism of extinction of a species is simply

[1] Berry, E. W. (1917[2]). [2] Willis, J. C. (1915). [3] Willis, J. C. (1916).

[4] The contention that Willis uses the word 'common' in a sense unusual among naturalists, is supported by the fact that Darwin (Origin of Species, 6th ed., 1894, p. 40) defines the most common species as those that 'abound most in individuals', and also draws a distinction between 'wide range' and 'commonness'.

[5] Willis, J. C. (1917) (the italics are mine). [6] Willis, J. C. (1918).

the converse of its mechanism of development, and hence consists in a progressive reduction of the area occupied? If we follow this assumption to its logical conclusion, we must suppose that each species in dying out steadily reduces its area, until it finally expires at its birthplace! This seems to be a fallacy comparable with the prevalent idea that structural degeneration is 'an actual retracing of steps until the point of departure is reached'.[1] There are, it is true, certain instances in which the area occupied by a dying species shrinks from the margin inwards; the Oxlip in East Anglia is an example,[2] but such cases are probably wholly exceptional. This plant (*Primula elatior*, Jacq.) occupies a restricted region and is surrounded on all sides by Primroses (*Primula vulgaris*, Huds.), which do not penetrate into its area. Year by year the Oxlip and Primrose hybridize on the margin of the Oxlip's demesne; it seems that the Oxlip will ultimately be hybridized out of existence, and its place taken by the Primrose, which is present in such hordes that the loss which it suffers by hybridization is negligible. It appears to me, however, that, apart from such rare cases as this, the tangible evidence of the dying out of a species would more probably be a progressive decrease in 'commonness on the ground', while the total area over which the species spreads might remain unchanged almost to the last. This gradual decrease in abundance would be a subtle matter to gauge and to define, but it ought to come within the scope of ecological observations, when the progress of that branch of Botany has enabled connected records to be kept over a long series of years. Willis's methods, on the other hand—valuable as they are for their own purpose—are not adapted for giving any indication of the progress of extinction, if it takes place in such a way that the total area from which the species is known remains unaltered.

For the reasons which I have attempted to outline, I consider that—although Willis's statistics undoubtedly substantiate the truth of his main hypothesis regarding Age and Area—they have no necessary bearing upon the question of the extinction of species.

BALFOUR LABORATORY,
CAMBRIDGE.

---

[1] There is a useful discussion of this and related questions in Demoor, J., Massart, J., and Vandervelde, E. (1899).

[2] Christy, M. (1897).

Literature cited.

Berry, E. W. (1917 [1]): A Note on the 'Age and Area' Hypothesis. Science, vol. xlvi, 1917, pp. 539–40.

———— (1917 [2]): Geologic History indicated by the Fossiliferous Deposits of the Wilcox Group (Eocene) at Meridian, Mississippi. U.S. Geol. Surv., Prof. Paper 108 E, Shorter Contributions to General Geology, 1917, pp. 61–72, 3 pls., 1 text-fig., 1 map.

Christy, M. (1897): *Primula elatior* in Britain: Its Distribution, Peculiarities, Hybrids, and Allies. Journ. Linn. Soc. Bot., vol. xxxiii, 1897–8, pp. 172–201, 1 map.

Demoor, J., Massart, J., and Vandervelde, E. (1899): Evolution by Atrophy. Trans. by Mrs. Chalmers Mitchell. Intern. Sci. Ser., vol. lxxxvii, 1899.

Ridley, H. N. (1916): On Endemism and the Mutation Theory. Ann. Bot., vol. xxx, 1916, pp. 551–74.

Sinnott, E. W. (1917): The 'Age and Area' Hypothesis and the Problem of Endemism. Ann. Bot., vol. xxxi, 1917, pp. 209–16.

Willis, J. C. (1915): The Endemic Flora of Ceylon, with Reference to Geographical Distribution and Evolution in General. Phil. Trans. Roy. Soc., Lond., Ser. B, vol. ccvi, 1915, pp. 307–42, 4 text-figs.

———— (1916): The Evolution of Species in Ceylon, with reference to the Dying Out of Species. Ann. Bot., vol. xxx, 1916, pp. 1–23, 2 text-figs.

———— (1917): The Relative Age of Endemic Species and other Controversial Points. Ibid., vol. xxxi, 1917, pp. 189–208.

———— (1918): The Sources and Distribution of the New Zealand Flora, with a Reply to Criticism. Ibid., vol. xxxii, 1918, pp. 339–67.

Studies on the Chloroplasts of Desmids. I.

BY

NELLIE CARTER, M.Sc.

With Plates XIV–XVIII.

CONTENTS.

	PAGE
I. INTRODUCTION	215
II. GENERAL CHARACTERS OF THE CHLOROPLASTS	219
III. THE CHLOROPLASTS OF *Netrium*	227
IV. THE CHLOROPLASTS OF *Closterium*	228
V. THE CHLOROPLASTS OF *Tetmemorus*	236
VI. THE CHLOROPLASTS OF *Euastrum*	237
VII. THE CHLOROPLASTS OF *Xanthidium*	245
VIII. SUMMARY	248

I. INTRODUCTION.

Historical.

THE first figures of Desmids which give one any clear idea of the nature of the cell-contents were those of Ralfs (1848). Although Ralfs did not attempt to indicate the form of the chloroplast in the larger species of *Euastrum* and *Cosmarium*, yet from his figures of such genera as *Desmidium*, *Closterium*, and *Staurastrum*, a fairly good idea of the structure of the chloroplasts in these forms can be obtained.

Nägeli (1849) also figured typical chloroplasts of the genera *Pleurotaenium*, *Closterium*, *Netrium*, *Staurastrum*, and *Cosmarium*.

De Bary (1858) described in general the chloroplasts of the group, and made a statement to the effect that all Desmids, with the exception of species of *Pleurotaenium* and *Spirotaenia*, have axile chloroplasts. This is by no means true, however, since parietal chloroplasts are known to occur in many other genera besides these two. De Bary also described in more detail some of the simplest types of chloroplast found in each genus, and gave an account of the behaviour of the chloroplasts in a few genera during cell-division.

Lundell (1877) attempted to subdivide the genera *Cosmarium* and *Staurastrum* according to the disposition of the chloroplasts, and instituted

[Annals of Botany, Vol. XXXIII. No. CXXX. April, 1919.]

two new sub-genera, *Pleurotaeniopsis* and *Pleurenterium*, to include those species of *Cosmarium* and *Staurastrum* respectively which have parietal chloroplasts. *Cosmarium* and *Staurastrum*, in the restricted sense of Lundell, contained only those species with axile chloroplasts.

The figures given by Delponte (1873) for such genera as *Desmidium* and other filamentous Desmids give one quite a good idea of the structure of the chloroplasts, whilst in many species of *Closterium*, *Penium*, *Netrium*, *Staurastrum*, and several species of *Cosmarium*, the form of the chloroplast is also indicated.

Klebs (1879) illustrated the ridged nature of the chloroplasts and the position of the pyrenoids in many species of *Closterium* and *Penium*, and also gave a figure of *Cosmarium de Baryi*, Arch., which is quite comparable with that given some years later by Lütkemüller (1893).

Schmitz (1882), in his work *Die Chromatophoren der Algen*, only just mentions Desmids as having chloroplasts of very diverse forms, which usually consist of a central axile portion provided with radiating plates of various kinds.

Gay (1884) figured the chloroplasts in a few species of *Closterium*, *Penium*, and *Micrasterias*, and also in species of several other genera.

An attempt to subdivide the genus *Xanthidium*, similar to that of Lundell (1871) in connexion with *Cosmarium* and *Staurastrum*, was made by Boldt (1888), who included in a new sub-genus, *Euxanthidium*, all species of the genus having parietal chloroplasts, and in *Centrenterium* all species in which the chloroplasts are axile. Boldt described a new species of *Xanthidium*, *X. groenlandicum*, Boldt, which he placed in the sub-genus *Centrenterium*, evidently believing it to have axile chloroplasts. The same species has been figured again by Larsen (1907), and although these figures are not particularly good for the chloroplasts, they do indicate, together with his description, that the chromatophores in this species are really very similar to those of other species of the genus which have parietal chloroplasts.

Lagerheim (1888) raised the sub-genera *Pleurotaeniopsis* and *Pleurenterium* of Lundell to the rank of genera.

Lütkemüller, who did some careful work on Desmids, published in 1893 a detailed account of the chloroplasts in certain species of *Cosmarium*, and also in a few other species formerly placed in Lagerheim's genus *Pleurotaeniopsis*. This was followed in 1895 and 1903 by a complete review of the genus *Spirotaenia*, and in this work the obscure structure of the chloroplast in those species belonging to the Polytaeniae was for the first time elucidated, and it was definitely shown that in these species of *Spirotaenia* the chloroplasts are really axile, in spite of the fact that the genus was originally supposed to be characterized by the fact that its chloroplasts are in the form of spirally twisted parietal bands.

These investigations of Dr. Lütkemüller may be regarded as the only

thorough work that has ever been done on the chloroplasts of Desmids, with the single exception of the more recent work of Lutman (1910, 1911) on *Closterium*. Lütkemüller was one of the few investigators who attempted to discover the real nature of the more complicated chloroplasts of the group. He worked for a considerable time on Desmids, and made many excellent preparations of the chloroplasts, but his untimely death in 1912 prevented this work from being published.

In their Monograph of the British Desmidiaceae, W. and G. S. West (1904–11) indicated the nature of the chloroplasts and the position of the pyrenoids in many members of the order, as far as this could be ascertained by superficial observation, noting in particular the variability of the chloroplast in *Cosmarium Subcucumis*, Schmidle, and other species. The same authors (1896, 1897, 1902, 1903, 1907) and G. S. West (1914) have figured the chloroplasts in *Roya*, W. and G. S. West, and also in some new species of *Cylindrocystis*, *C. Americana*, W. and G. S. West,[1] *C. obesa*, W. and G. S. West, and *C. pyramidata*, W. and G. S. West,[1] besides indicating the nature of the chloroplasts in several other species.

Seeing that there is no exact information concerning the nature of the chromatophores in the great majority of Desmids, it was suggested by Professor G. S. West that the investigation of the chloroplasts of this group would be a useful and interesting study.

Methods.

With most Desmids, careful staining is essential for the investigation of the chloroplasts; only in a very few species of *Staurastrum*, *Cosmarium*, and *Closterium* can the nature of the cell-contents be successfully determined in the living condition, and even in these staining is desirable in order to ascertain the number and position of the pyrenoids. In all others the contents are uniformly green in the living condition, or merely show a few darker bands, their real nature being quite obscure. With some of the thicker species, even in stained specimens it is quite impossible to make out with any certainty the structure of the chloroplasts, because of the extremely dense nature of the cell-contents, and sections are essential, as for example in the larger species of *Euastrum* and *Cosmarium*. In the investigation of the chloroplasts of the large species of *Euastrum* particularly, sections revealed the most unexpected structure, and it is quite probable that their complicated construction would never have been understood by mere superficial observation. The same is most certainly true in the case of many other large Desmids, and with these also sectioning is the only means of obtaining accurate information.

In the case of *Cosmarium biretum*, Bréb., the cell-wall appeared to have a peculiar affinity for the stain used, iron-haematoxylin staining quite

[1] It is most probable that these two species really belong to the genus *Cosmarium*.

black, and entirely obscuring the cell-contents in whole mounts. Naturally it was quite impossible to get any idea of the structure of the chloroplast from such preparations, and consequently it was absolutely necessary to cut sections. The same phenomenon has been noticed, though not to the same extent, in a few other species of *Cosmarium*, and also in certain species of *Closterium* and *Penium*. On the whole, however, it is quite unusual for the cell-wall to absorb the stain in this way.

In the case of those species of *Closterium* and *Penium* whose cell-walls seem to have such a marked affinity for haematoxylin, this is probably to be attributed to the presence of iron salts in the composition of the wall, which help to fix the stain. The same explanation, however, is not true in the case of *Cosmarium biretum*, since a test for iron in its cell-wall gave only negative results. In the latter species it is possibly the presence of considerable quantities of some other mineral salt, probably lime, which accounts for the deeply staining properties of the cell-wall.

When the cell-wall in *Closterium* becomes very old and brown, the power of attracting the haematoxylin seems to be lost, and the cell-wall scarcely stains at all. This is possibly due to the fact that the iron probably becomes united with a large organic radical, in which complex form it is incapable of acting as a mordant.

A large part of the work was done from fresh material collected chiefly in Sutton Park, Warwickshire, and in Devonshire, but a considerable number of species were investigated from material furnished by Professor G. S. West, which had been preserved for some time in formalin or potassium copper acetate. After well washing such material in water, the structure of the chloroplast was usually quite clear on staining.

The investigation of a few species was carried out from some preparations made by Dr. Lütkemüller, and very kindly lent by Professor G. S. West. The specimens had been stained in Delafield's haematoxylin and were mounted in Venetian turpentine.

The living material was always fixed as soon as possible after the collections had been made, because most species do not live long when removed from their natural habitat. The most satisfactory fixing reagent was found to be a hot solution of corrosive sublimate. Bouin's picric-formol-acetic fixing solution always produces considerable shrinkage of the cell-contents, even when very much diluted, and with Flemming's weaker solution the staining is rarely so satisfactory. The solution used consisted of corrosive sublimate, 3 grm.; glacial acetic acid, 3 c.c.; 50 per cent. alcohol, 100 c.c.

The hot sublimate was removed from the material as soon as possible and replaced by clean 50 per cent. alcohol. After washing in several changes of alcohol the remaining sublimate was removed by the addition of a dilute alcoholic solution of iodine. The material was then taken either

down into water for staining, or up through absolute alcohol and xylol, and finally into paraffin for sectioning, the transference to the various liquids being made as gradual as possible. A centrifuge was always used for facilitating the removal of solutions, and thus it was possible to transfer the material without loss, and in a comparatively short time, through the numerous different strengths of solutions which were absolutely necessary in order to prevent the shrinkage of the cell-contents. In transferring from water to absolute alcohol and xylol, the material could be taken without shrinkage through solutions of strengths increasing successively by 10 per cent., being kept about 10 minutes in each one. It would have been quite impossible to get through so much material if it had been necessary to wait for the Desmids to settle by gravity each time the solution was changed.

The most satisfactory stain was found to be Heidenhain's iron-haematoxylin. This always gives a perfect differentiation of the chloroplasts, the general cytoplasm staining very faintly or not at all. Material was transferred from water to a $2\frac{1}{2}$ per cent. solution of iron alum, and allowed to remain in this for 10 to 20 minutes. After rinsing in water it was stained for about 40 minutes in $\frac{1}{2}$ per cent. aqueous solution of haematoxylin. The proper differentiation of the cell-contents was obtained by washing out part of the stain in diluted mordant, the operation being controlled by watching a drop of the material under the microscope. After well washing in water the material was transferred gradually to absolute alcohol and xylol, and finally placed in very dilute Canada balsam, which was allowed to concentrate slowly until it was of a consistency suitable for mounting.

Venetian turpentine was also used instead of Canada balsam with equally good results. The material was transferred from 95 per cent. alcohol to the diluted turpentine, and allowed to concentrate in an atmosphere kept dry by means of fused calcium chloride.

II. General Characters of the Chloroplasts.

In the lower group of the Desmidiaceae the form of the chloroplasts is usually very much simpler than in the Placodermae, the only genera in which very complicated structure occurs being *Netrium* and *Spirotaenia*. Consequently the chloroplasts of these rather more primitive Desmids are very much better known. Thus the simple axile chloroplasts of *Gonatozygon* and *Mesotaenium*, and the spirally-twisted bands of *Genicularia*, were figured by such early investigators as Nägeli (1849) and de Bary (1858). The chloroplasts in two species of *Spirotaenia* were figured by Ralfs (1848), and the whole genus has since been thoroughly investigated by Lütkemüller (1895), whilst in the case of *Roya* the chloroplast has been figured by W. and G. S. West (1896). In all these genera the chloroplasts usually stretch from end to end of the cell, and the nucleus often occupies in con-

sequence a more or less lateral position, but in some individuals the chloroplast may be interrupted in this region, so that two chloroplasts are present instead of one. In the spirally twisted bands of *Genicularia* and the parietal chloroplasts of some species of *Spirotaenia* the pyrenoids are scattered in the chloroplast near the cell-wall, but in all others they lie in a single row in the middle of the cell, and in *Mesotaenium* there may be only one pyrenoid in the central position.

Cylindrocystis and *Netrium* differ from all the other Saccodermae in the somewhat complicated form of their chloroplasts (Figs. 13–19). In both genera there are invariably two chloroplasts in each cell, each one consisting of a central axile mass containing typically one pyrenoid and a number of strands or plates which radiate towards the periphery. The chloroplasts of these two genera have been figured by de Bary (1858) and West (1902; 1904–11, vol. i). *Netrium interruptum*, (Bréb.) Lütkem., is unique amongst the Saccodermae in the possession of four axile chloroplasts in each cell, one above the other in a longitudinal series.

Amongst the Placodermae there is much greater variety in the form of the chloroplasts, and, with the exception of some filamentous species, most of them have hitherto never been figured. There is almost invariably in the higher Desmids at least one chloroplast in each semi-cell, although in a few very small species, e.g. *Cosmarium subtile*,[1] (West) Lütkem., *C. tinctum*,[2] Ralfs, *C. subtilissimum*,[3] G. S. West, *Cosmocladium constrictum*,[4] Arch., and *Staurastrum inconspicuum*,[2] Nordst., there is only one simple chloroplast in each cell, typically with a single pyrenoid in the central position. In the larger species of the group the chloroplasts may be axile or parietal. When axile there may be either one chloroplast in the centre of each semi-cell, or else two placed side by side. In *Closterium Libellula*, Focke, var. *interruptum*,[5] W. and G. S. West, there are two axile chloroplasts in each half-cell, one on top of the other, just as in *Netrium interruptum*. When parietal there may be four or more chloroplasts in each semi-cell, and here there is far more variation in the actual number of chloroplasts present than is the case with axile ones. In species which normally have two axile chloroplasts in each semi-cell, more or less than two are rarely observed, and although in the case of species normally having one axile chloroplast in each semi-cell one sometimes encounters individuals which have two, this is quite unusual. In the case of parietal chloroplasts, however, the number of plates or bands present may vary from two to eight or even more.

[1] Vide W. and G. S. West (1904–11, vol. i). This species was figured under the name of *Penium subtile*.

[2] Vide Lütkemüller (1902), p. 356.

[3] Vide G. S. West (1914).

[4] Vide G. S. West (1904).

[5] Vide W. and G. S. West (1904–11, vol. i). This species was figured under the name of *Penium Libellula*, var. *interruptum*.

The most striking thing about the chloroplasts of the higher members of the Desmidiaceae is their complexity of form. It is very rarely that one finds straight outlines, or simple ridges; on the other hand, the chloroplasts are usually most complicated structures, the margins of the plates of which they are composed being divided and subdivided, and the ridges often branched repeatedly; cf. Figs. 65 and 77.

In many species which have greater or smaller parietal expanses of chloroplast, the main body of the chloroplast is often removed a short distance away from the cell-wall, and numerous short outgrowths of chloroplast extend from it towards the periphery. Such outgrowths were first noticed by Lütkemüller (1903), being described by him for *Cosmarium tessellatum*,[1] *C. turgidum*, and *C. de Baryi*. It is most probable, however, that the phenomenon is not confined to these species, but is quite general, occurring in most genera where parietal or partly parietal chloroplasts are to be found. It has been observed during the present investigation in other species of *Cosmarium*, in various species of *Euastrum* and *Xanthidium* (see Figs. 62, 72, 89, 100, and 113), and also in *Pleurotaenium elephantinum*, Cohn.

On the other hand, these projections do not seem to be an absolutely constant feature of any species in which they may be observed, but probably depend to a considerable extent on the condition of the cell. They are often very conspicuous in individuals with massive chromatophores, being in such cases both numerous and of large size. In cells with feebly developed chloroplasts they are often altogether absent, and the parietal part of the chloroplast closely envelops the cell-wall. In general it is in cells with much available chlorophyll-bearing material that the most elaborate chloroplasts are to be found, not only with regard to these projections from the external surface of the parietal parts, but also in the branching of the ridges and the elegance of the lobing and toothing of the edges of the various plates. But even in specimens with well-developed chloroplasts, the delicate form of the chromatophore may be secondarily interfered with by the presence of large quantities of starch, as will be explained later.

Usually in cells with feebly developed choroplasts the same general structure can be recognized as in the better developed specimen of the same species, except that all superfluous ornamentation is wanting. Sometimes, however, a considerable difference in appearance may be presented by two specimens of the same species, one with well-developed chloroplasts, the other with very little photosynthetic material. This is very marked in *Cosmarium Brebissonii*, Menegh., where some individuals have such large chloroplasts that these penetrate every part of the cell, which is almost full of pyrenoids and chloroplasts, whilst other specimens contain so little chlorophyll-bearing material that the chloroplasts form merely a thin layer lining the cell-wall, the interior of the cell being quite free from them.

[1] Lütkemüller described these three species under the generic name of *Pleurotaeniopsis*.

Thus it is possible for considerable variation to occur in the form of the chloroplast within the limits of a single species, according to the relative amount of chlorophyll-bearing material present.

The general appearance of the chloroplast seems to be also greatly influenced by the amount of starch contained in it as free stroma-starch apart from that occurring in immediate connexion with the pyrenoids. This difference can sometimes be distinguished in the living condition, but it is much more evident after staining.

Some individuals stain much more deeply than others, whilst at the same time the form of their chloroplasts is much more definite, the various plates of which they are composed being very distinct and sharply defined, their pyrenoids are conspicuous as dark globules with very definite starch-sheaths, and on examining the more minute structure of the chloroplast its protoplasmic reticulum is seen to be very fine and close in texture, its whole substance appearing homogeneous, except for the pyrenoids.

On the other hand, certain individuals present a very different appearance. Their chloroplasts do not stain nearly so deeply and are much more bulky, not having the fine definition of the first type, whilst the distinction between chromatophores and general cytoplasm is not nearly so marked as before. Although there is a paler region round each deeply staining pyrenoid, a really definite starch-sheath cannot be distinguished, since there is a gradual transition to the general substance of the chromatophore, which, when closely examined, is seen to have a very granular appearance, instead of being practically homogeneous as before.

On testing with iodine it is found that the first type of individual contains practically no starch except that which forms the starch-sheaths of its pyrenoids, whilst in the second type the chloroplasts are absolutely packed throughout with starch, and it is the presence of innumerable starch-grains which causes the granular appearance of the chromatophore described above. It seems that under certain conditions starch-grains are thrown off from the pyrenoids in large numbers, and spread into every part of the chromatophore, stretching apart the meshes of its fine protoplasmic reticulum and causing the chloroplast to swell to three or four times its former bulk. During the process of becoming swollen with stroma-starch the chloroplast often naturally loses its delicate form and becomes a comparatively shapeless mass. As a direct result of this, the more delicate parts of its structure, e.g. the numerous finger-like projections sometimes found on the external surface of many parietal chloroplasts, may be partly or entirely obliterated. The distending of the chloroplast begins in the region of the pyrenoids and gradually extends to the more remote parts. This phenomenon sometimes causes remarkable variations in the form of the chloroplast, particularly in certain species of *Closterium*, in which the relative size of the central axis and radiating ridges is largely dependent on the amount of starch present.

In some species the form of the chloroplast varies very little amongst individuals, and a typical form can be described which is true in all essentials for practically all specimens, the only differences being minor ones, such as those due to external conditions, as mentioned above. This is the case with many species of *Staurastrum* and *Cosmarium*. In other species there is a marked tendency to vary, and a considerable proportion of individuals do not conform to the type. This is very noticeable in the larger species of *Euastrum*, where, although the typical form of the chloroplast is axile, all stages to the parietal condition are to be found; cf. Figs. 72–5 and 100–5. Lütkemüller (1893) noticed the existence of such variations in *Cosmarium docidioides*[1] and *Xanthidium Brebissonii*,[2] and possibly it also occurs in other species. The chloroplasts of the thicker-celled species of *Micrasterias* are also variable, but in quite a different way.

The very intimate connexion between the nucleus and chloroplasts was noticed in many species. This is seen best where there are two axile chloroplasts in each semi-cell, as in certain species of *Cosmarium*. In such cases the basal part of each chloroplast is drawn out into a string-like portion, which is apparently attached directly to a corner of the nucleus, this being usually more or less rectangular in shape. Very often as a result of the frequent use of the centrifuge in the preparation of the material, the nucleus becomes displaced from its normal position in the isthmus, and is to be seen a little distance away in one of the semi-cells, dragging behind it the chloroplasts by means of their string-like attachments, the connexion between them still unbroken. Thus there must be a very vital connexion between the nucleus and chloroplasts, either directly or through the medium of the layers of protoplasm which surround them. In any case it must be very strong to withstand the treatment described above.

Sometimes similar thread-like attachments are noticed between the edges of the chloroplast plates and the layers of cytoplasm lining the cell-wall. This seems to be very common with chloroplasts which are not sufficiently massive to extend right up to the cell-wall at all points, especially in the case of young semi-cells.

The number and position of the pyrenoids vary considerably amongst different individuals of the group, depending to a great extent on the shape of the chloroplast, their number being also bound up with the general condition of the cell. In most species which have large parietal chloroplasts the pyrenoids are indefinite and are scattered indiscriminately (Figs. 102 and 129). Some species, e.g. small species of *Xanthidium*, have smaller parietal plates, each of which has typically one pyrenoid (Figs. 106, 109, 113, and 115).

[1] Lütkemüller described the chloroplasts of this Desmid under the name of *Docidium baculum*.

[2] That Lütkemüller also noticed the variation of the chloroplasts in this species is indicated in some of his unpublished drawings.

In the case of species having axile chloroplasts, the pyrenoids are usually found in those parts of the cell where the chloroplast is most massive. This is frequently the axis of the chloroplast itself, but in some cases, e.g. the larger species of *Euastrum*, the actual axis of the chloroplast is very slender, and the pyrenoids are practically confined to the more massive peripheral parts of the chloroplast (Fig. 73). The number of pyrenoids embedded in the axis of axile chloroplasts depends on its size. Thus where there is a long narrow axis, e.g. *Closterium*, the pyrenoids occur in a line, and may be quite numerous (Figs. 6, 10, 23, 35, 38, and 47). Where the axis is larger and broader, e.g. some species of *Micrasterias*, the number of pyrenoids present may be more than 100, and they are scattered throughout. In many species where the axis is much more limited in its extent, there is typically only one pyrenoid in the axis of each chloroplast.

The general condition of the cell, however, probably greatly influences the amount of food-reserves stored in the form of pyrenoids, and at any time two or more pyrenoids may occur in such positions where only one would have been expected. This is true both in the case of axile and parietal chloroplasts, which were formerly supposed to have only one pyrenoid.

When division of a pyrenoid occurs, usually by budding, or the gradual constriction of one to form two, the newly formed pyrenoids separate from each other almost immediately, where this is possible, e.g. the extensive chloroplast-plates of *Xanthidium armatum*, (Bréb.) Rabenh. (Figs. 129 and 130), or *Micrasterias denticulata*, Bréb. Where the space is more limited, however, the migration of the pyrenoids is impossible. For example, some species of *Cosmarium* have two axile chloroplasts in each semi-cell, each chloroplast containing originally a single pyrenoid. If either of these pyrenoids divides, the products of its division could not possibly separate, because the chloroplast only forms a minute film round it, and the two pyrenoids remain in the position of the original one. Further division may result in the formation of a group of three or even more pyrenoids in the axis of one or more of the chloroplasts in some individuals. In the living condition such a group of pyrenoids would be apparent simply as one refractive mass, and it would be almost impossible to distinguish it from a single pyrenoid. This is probably why the number of pyrenoids has been thought to be definite in so many Desmids. The examination of stained material, however, has shown that this is not so, although the relative position of the pyrenoid groups is really quite constant in such cases.

Lütkemüller (1893) noted such variations in the number of pyrenoids in several species of *Cosmarium*, and also in species of *Arthrodesmus*, *Staurastrum*, and *Euastrum*, whilst W. and G. S. West (1898) have also

reported unusually large numbers of pyrenoids in *Hyalotheca neglecta*, Racib., and *Cosmarium sphagnicola*, W. and G. S. West.

The observations of these investigators are supported by Ducellier (1917), who noted some irregularities in the number of the pyrenoids in various species of the genus *Cosmarium*, and gave some evidence that the number of pyrenoids in this genus is not always one or two, as was formerly stated. The present investigation also supports this, not only with regard to the genus *Cosmarium*, but also to species of other genera which have chloroplasts containing typically one pyrenoid. The single pyrenoid may, under favourable conditions, give rise to a group of two or more pyrenoids, and so long as the group of pyrenoids occupies the same relative position as the original one, the specimen can be regarded as being quite ordinary; cf. Fig. 59. Of course one occasionally meets with individuals which have an abnormal number of pyrenoids in unusual positions. Thus specimens of *Euastrum pectinatum*, Bréb., *Eu. bidentatum*, Näg., *Cosmarium subtumidum*, Nordst., &c., have been encountered having two chloroplasts in each semi-cell, each containing one pyrenoid, whereas normally there should be only one chloroplast, containing one pyrenoid; cf. Figs. 51 and 54, 66 and 70. If the two pyrenoids had simply occupied the same position as if there had been only one, the specimens would have been regarded as normal, but since each pyrenoid was contained in a distinct and separate chloroplast, they were regarded as being unusual—and were omitted as such. Different anomalies were met with in other species.

Ducellier also raises the question whether the number of pyrenoids contained in any cell belonging to the genus *Cosmarium* is not dependent to some extent on the size of the individual. This does not seem to be the case, either in *Cosmarium* or in any other genus of Desmids. Ducellier does not show the chloroplast in any of his figures, and it would appear that this is a factor which he has entirely neglected. The actual mass of chlorophyll-bearing material contained in the cell undoubtedly does have a direct effect on the amount of food-reserves stored as pyrenoids, but the amount of chloroplast present in individuals of one species varies considerably, irrespective of their size. Thus in some large cells the chloroplast may be particularly scanty, whilst in smaller individuals of the same species it may be quite massive; in such cases the large cell probably contains less reserve food than the smaller one. Again, the conditions which control the division of the pyrenoid are very obscure, and cannot be determined. For in the same collection one individual may contain a few large pyrenoids, whilst another of the same species may contain many smaller ones. Furthermore, the conditions governing pyrenoid division may vary in different parts of the same semi-cell. For example, in one specimen of *Xanthidium Brebissonii* containing two axile chloroplasts in a semi-cell, one chloroplast was provided with a single extremely large pyrenoid, whilst

the other had a group of small ones (see Fig. 122). In view of these facts it seems very unlikely that there is any definite relation between the number of pyrenoids contained in a cell and its size.

The method of division observed in the pyrenoids was nearly always by constriction or pulling apart of one individual to form two. More rarely the pyrenoids divide by a clean cut into two parts, or the pyreno-crystal fragments to form many. In many cases when actively dividing the pyreno-crystal was observed in the process of budding off two or more new pyrenoids at the same time (Figs. 59, 60, and 80).

Whilst examining the chloroplasts of *Cosmarium ochthodes*, Nordst., numerous darkly staining globules were noticed, sometimes in the peripheral parts of the radiating plates, but more often in the reticulated films of chloroplast stretching from these over the interior of the cell-wall. In stained preparations they have the general appearance of small naked pyrenoids, staining in exactly the same way as the pyreno-crystal of the larger pyrenoids of the cell. They do not appear to have a definite starch-sheath, but are always surrounded by a lighter space. In sections stained with iodine the globules become distinctly brown, and when tested with other reagents they always give the same reaction as the pyreno-crystals of the ordinary pyrenoids. They stain brilliantly with acid fuchsin and are undoubtedly of a proteid nature.

Similar globules have been observed in several other species of *Cosmarium* and *Closterium* (Fig. 44), in one species of *Cylindrocystis*, and they have also been found scattered amongst the larger pyrenoids in the chloroplasts of *Micrasterias denticulata* and *Xanthidium armatum*. When tested in several of these other species they were found to behave in exactly the same way as before.

Their position in those parts of the chloroplast nearest the cell-wall is a constant feature, and is very marked in every case in which they were observed with the exception of *Micrasterias denticulata*, in which species this distinction is scarcely possible. They vary considerably in number and are not found in every individual of such species in which they have been observed.

They seem to have no relations with the ordinary large pyrenoids of the cell, and are probably not derived from them in any way, being most likely formed *de novo* when the conditions in the cell are favourable for the storage of large quantities of reserve food. In all the species of *Cosmarium* in which these small globules were observed, the points at which the ordinary pyrenoids occur are definite and fixed, usually in the interior of the cell, and even if these do divide the products of their division remain in the position of the original ones, whilst these small globules are only to be found near the cell-wall. Thus the possibility of their origin from the division of the ordinary large pyrenoids can be neglected.

The only record of similar pyrenoids occurring in the Chlorophyceae is provided by Schmitz [1] (1882), who reports that in some of the thicker-celled species of *Zygnema*, very small pyrenoids are sometimes apparent in the peripheral ends of the rays of the star-shaped chloroplasts. Schmitz does not definitely say that these pyrenoids were destitute of starch, but their position near the cell-wall and their supposed origin *de novo* is very suggestive of the small proteid granules observed in certain Desmids.

It is proposed to give, as far as is possible from the limited number of species examined, a comparative account of the chloroplasts found in each genus, excluding those genera in which the form of these bodies is already well known, and in this part of the work the genera dealt with will be *Netrium*, *Closterium*, *Tetmemorus*, *Euastrum*, and *Xanthidium*.

III. The Chloroplasts of the Genus *Netrium*.

This genus belongs to the Saccodermae, and the species examined were *N. Digitus*, (Ehrenb.) Itzigs. and Rothe, *N. oblongum*, (de Bary) Lütkem., *N. oblongum*, var. *cylindricum*, West and G. S. West, and *N. interruptum*, (Bréb.) Lütkem. The chloroplasts of these species are generally well known, and consist of a central axis from which a number of plates are given off. In *N. Digitus* these are nearly always eight in number (Fig. 15), in *N. oblongum* there are eight or nine, in *N. oblongum*, var. *cylindricum*, about seven (Fig. 18), and in *N. interruptum* about eleven or twelve (Fig. 19), but except in the case of *N. Digitus* the counting of the plates had to be done from whole specimens, and so these numbers may not be exact.

The peripheral edges of the plates in *N. Digitus* are more or less deeply cut into teeth which project alternately on either side of the plate towards the cell-wall (Figs. 13 and 15). The actual size and shape of the projections vary considerably; in some cases they consist of long fine strands which stretch in various directions towards the cell-wall, whilst in other individuals they are quite flat and angular.

In *N. oblongum* the plates are similarly notched but are not quite so complicated (Figs. 16 and 17), and in *N. interruptum* the edges of the plates are entire (Fig. 19). The latter species also differs from the others in having two chloroplasts in each half-cell.

The pyrenoids found in these species of the genus are peculiar in several ways. They occur only in the central axis of the chloroplast and are usually of extraordinary length, there being as a rule one pyrenoid only in each chloroplast, which is apparent as a conspicuous darkly staining rod in the middle of each half-cell (Fig. 14). The amount of starch round each pyrenoid naturally varies very much according to the condition of the cell, but the starch grains are always very small in comparison with the size of

[1] p. 74.

the pyreno-crystal, and it is only very rarely that there is only one layer of starch grains round each pyrenoid. In most cases the starch layer is of some thickness, and consists of very numerous starch grains closely packed together, either in a compact even layer or projecting irregularly in all directions into the reticulum of the chromatophore (Figs. 14, 16, 17, 18, and 19).

In some cases the pyreno-crystal is observed to fragment, forming several shorter, or very many small spherical or irregularly shaped globules in a longitudinal series embedded in the central cylinder of starch-grains (Fig. 17). The size of the small pieces of pyreno-crystal varies very much; they are often very irregular in shape and are not necessarily arranged in a single series in the middle of the chloroplast, two or more often occurring side by side.

Such complex pyrenoids have only been observed, outside the genus *Netrium*, in the chloroplasts of *Closterium Libellula*, Focke, *Penium spirostriolatum*, Barker (Fig. 20), *P. polymorphum*, Perty, *P. margaritaceum*, (Ehrenb.) Bréb., and *Cylindrocystis Brebissonii*, Menegh.

In *Netrium interruptum* one specimen was observed to have only one chloroplast in each half-cell, with a single pyrenoid stretching throughout its whole length (Fig. 19). There were, however, signs of the constriction of the chloroplast about half-way between the nucleus and the apex of the cell. This suggests that for a time after cell-division each individual has only one chloroplast with one pyrenoid, and that the division of this to produce the two usually present does not occur until some time afterwards.

IV. The Chloroplasts of the Genus *Closterium*.

In 1910 Lutman published a paper describing the chloroplasts of *Cl. Ehrenbergii* and *Cl. moniliferum*, figuring transverse sections of both species, and pointing out how the structure of the chloroplast in the genus *Closterium*, as he found it in these two species, differs from that figured by Nägeli (1849) for *Cl. parvulum* and *Cl. moniliferum*. According to Lutman, the chloroplast of *Closterium* in transverse section is like a coarsely cogged wheel, whereas according to Nägeli's figures it is like a hub with radiating spokes. Lutman asserted that Nägeli's optical sections give quite an erroneous conception of the true state of affairs, the chloroplast in *Closterium* consisting of a curved cone-shaped structure with relatively low ridges on its surface, and not of a series of radiating plates arranged round a slender central core.

With the object of ascertaining whether the examination of other species of *Closterium* would support Lutman's statement, the chloroplasts of the following species have been investigated, both by means of whole mounts and of sections:—*Cl. Lunula*, (Müll.) Nitzsch., *Cl. lanceolatum*, Kütz.,

Cl. Siliqua, West and G. S. West, *Cl. costatum*, Corda, *Cl. striolatum*, Ehrenb., *Cl. regulare*, Bréb., *Cl. angustatum*, Kütz., *Cl. attenuatum*, Ehrenb., *Cl. Libellula*, Focke, *Cl. Dianae*, Ehrenb., *Cl. rostratum*, Ehrenb., and *Cl. juncidum*, Ralfs. This has led to the conclusion that the broad axis figured by Lutman for *Cl. Ehrenbergii* and *Cl. moniliferum* is not common to all species of the genus, and furthermore that even within the limits of a single species the relative size of the axis and ridges cannot be considered as a fixed character, but is, on the other hand, liable to considerable individual variation, according to the condition of the cell, this being, however, more pronounced in certain species than in others.

In a few of the species examined, e.g. *Cl. Lunula*, *Cl. lanceolatum*, and *Cl. Siliqua*, the structure of the chloroplast as seen in transverse section is often quite similar to that figured by Lutman, but even here the axis is sometimes much more slender in proportion than his figures show it (Figs. 2–5). *Cl. attenuatum* and *Cl. Libellula* seem almost invariably to have chloroplasts, which in transverse section are rather more like Nägeli's figures than those of Lutman, in having fairly large radiating plates and a comparatively slender axis (Figs. 9 and 12).

In *Cl. costatum*, *Cl. striolatum*, and *Cl. regulare*, however, the most conspicuous variations in the size of the central axis occur, transverse sections of some individuals of these species being quite similar to those figured by Lutman (Figs. 30 and 34), whilst sections of other specimens may present the most unexpected appearance (Figs. 26–9 and 40–2). The axis in these peculiar chloroplasts may quite lose its definite shape as a more or less cone-shaped structure, the whole chloroplast in transverse section being irregularly branched, so that the axis appears merely as a strand of tissue connecting up the irregularly arranged ridges (Fig. 41). This strand of material, which constitutes the axis, may be very much swollen at intervals for the accommodation of the pyrenoids, but it is usually very drawn out in between.

In all the species examined, with the exception of *Cl. Lunula* (Figs. 1–5), the pyrenoids occur in a single row in the middle of the central axis, but when photosynthesis has been going on very rapidly, and the pyrenoids have become very numerous, they occasionally spread in certain places towards the periphery; cf. Fig. 34. In *Cl. Lunula* the relative position of the pyrenoids depends on the size of the axis of the chloroplast. They are usually embedded in the peripheral parts of the axis; thus in cells having a broad axis and low ridges they are quite near the cell-wall, and in such cases are never found very far in the interior of the cell (Figs. 1 and 2), whilst in other individuals having a very slender axis and large ridges, the pyrenoids are crowded together in the comparatively small axis of the chloroplast, which in such cases may be an almost solid mass of pyrenoids and starch (Figs. 3 and 4). This variation in the size of the axis and the

relative position of the pyrenoids has also been noticed in *Cl. Ehrenbergii*, although it is not mentioned by Lutman (1910).

The number of ridges arranged round the central axis is fairly constant for each species, *Cl. striolatum* having twelve or thirteen, *Cl. angustatum* nine or ten, and so on.

The edges of the ridges are only ornamented to any great extent in *Cl. Lunula*. In this species they are hollowed out at intervals, or regularly lobed throughout their whole length, and these lobes or projections stretch out towards the cell-wall (Figs. 1 and 3). The edges of the ridges in *Cl. Libellula* are usually conspicuously sharp and straight.

The size of the ridges, as has already been stated, is a very variable character, and in certain species this variability may greatly influence the external appearance of the individual. In many cases the ridges when examined from the exterior are apparent as nearly parallel dark lines stretching from the region of the nucleus to the apices of the cell. This is usually the case in *Cl. Libellula* (Fig. 8). In certain individuals, however, particularly of the species *Cl. costatum*, *Cl. striolatum*, *Cl. regulare*, and *Cl. angustatum*, the ridges seem to travel in tortuous paths from end to end of the cell, and sometimes appear to lose their individuality by apparently fusing for a short distance one with another (Figs. 31 and 38). Although this is a very common occurrence in all the four above-mentioned species, it is by no means a constant feature of any one of them, individuals having ordinary straight ridges being also sometimes met with. Thus in some individuals of these species the ridges may appear to undulate very gently, and in extreme cases each individual ridge may bend alternately to the left and to the right in such a way as to produce a reticulation something like a honeycomb, or more irregular anastomosis against the interior of the cell-wall.

This irregular arrangement of the ridges has been figured by Delponte (1873) in *Cl. didymotocum*,[1] and also in two other species described by him as *Cl. hirudo*[2] and *Cl. crassum*.[3] In the case of the first species at any rate he described the chloroplast as consisting of tortuous strings and tubules immersed in the protoplasm, and seemed very uncertain about the existence in this species of a chloroplast consisting of lamellae radiating from the centre to the periphery. Although *Cl. didymotocum* was not examined during this investigation, there seems to be no doubt that the appearance figured by Delponte was due to the same anastomosing of the ridges as has been observed in *Cl. costatum*, &c., in which species it has been definitely proved that the superficial tortuous ribbons such as were described by him for *Cl. didymotocum* are really joined up to the axis of the chloroplast in the interior of the cell, and are not merely isolated strings, as he seemed to think.

[1] Delp., l.c., Tav. XVII, Figs. 32–4.
[2] Delp., l.c., Tav. XVIII, Fig. 7.
[3] Delp., l.c., Tav. XVIII, Fig. 23.

In the case of specimens having nearly parallel or very slightly undulating ridges, these are seen in transverse section radiating from the central axis at practically equal distances from each other all the way round, and as a rule the ridges in such cases are very small in comparison with the size of the axis (Figs. 30 and 34). But where the undulation of the ridges becomes very pronounced, and their paths are very tortuous, transverse sections reveal a remarkable difference of structure. The axis here is usually very slender, and the ridges, instead of originating quite independently of each other from the axis, as in cases where they are nearly parallel, are seen in transverse section to show a certain amount of branching. Thus in the case of an individual of *Cl. costatum*, which would have had fourteen ridges visible from the exterior, a transverse section showed that the central axis gives off five ridges only, and that the fourteen ridges lying against the cell-wall are the ultimate products of the branching of these original five (Fig. 32). Another specimen of the same species had thirteen ridges resulting from the subdivision of four primary ridges, and so on, the arrangement of the ridges round the axis varying with individuals.

Again, the combination of the ridges at various points in the same cell differs continually, as the examination of consecutive sections shows. Thus Figs. 41–3 represent consecutive sections of a specimen of *Cl. striolatum* having thirteen ridges, and from a comparison of these the rapidly changing position and arrangement of the individual ridges in a short length of the cell is quite evident. The ridges, where they overlap the nucleus (Fig. 43), are quite free and distinct from each other, whilst in the remaining two sections the ridges are joined together in quite different combinations in each case. Thus it is clear that any particular ridge partially fuses for a greater or shorter distance with one or more neighbouring ridges, and, at various points in the length of the cell, any individual ridge is united with ever-varying groups of other ridges, being restricted of course to its own immediate neighbours.

As seen in transverse section, however, the fusion of any two ridges rarely extends all the distance from the central axis to the periphery, the ridges being quite free from each other as they lie against the cell-wall, but fusing in various combinations in the interior of the cell (Figs. 29, 32, and 41). Where, in the external view of an individual, two ridges come so close together as apparently to fuse, a careful examination will usually show that they are quite distinct from each other on the surface (Fig. 38), although they are probably united just beneath, as seen in the tangential section, Fig. 39. Sometimes, however, complete fusion of two ridges may occur near the apex of an individual, or one ridge may end abruptly, so that there may not be so many ridges round the axis near the apex of the cell as lower down.

Thus, throughout the whole length of the cell the arrangement of the

ridges round the central axis in these complicated chloroplasts is continually changing, the position of any particular ridge in relation to the other varying from time to time, and it is this which probably accounts for the repeated bringing together and separation of neighbouring ridges which causes the reticulate appearance seen in the external view.

In species like *Cl. costatum*, *Cl. striolatum*, *Cl. regulare*, and *Cl. angustatum*, therefore, transverse sections of different individuals may present striking differences of structure, according to the relative size of the ridges and axis. If the ridges of the individual in the external view are nearly parallel, then in section the axis will probably be very broad, occupying most of the interior of the cell, whilst the ridges will be comparatively low, but quite distinct from each other. If, on the other hand, the ridges are anastomosing to any great extent, in such cases the axis in transverse section will be much smaller, the ridges will be more extensive and also more or less branched, the chloroplast as a whole being much more irregular in outline.

As regards the finer structure of the chloroplast, it was noticed that in sections of *Closterium* showing branched ridges the protoplasmic network of the chloroplast is much closer and more compact than that of sections showing a broad axis and low ridges. This is particularly noticeable in those species in which there is the possibility of very great variation in the relative size of the axis and ridges, those specimens with large or branched ridges showing an extremely fine reticulation of the chloroplast, and moreover the ridges in such cases are usually very thin, but the protoplasmic network of the whole chloroplast is very dense and very sharply defined from the rest of the protoplast (Figs. 26–9, 32, and 41–2). In specimens of these species which have low ridges and a very massive central axis, the chloroplast is seen in transverse section to have a very coarsely reticulate appearance, which on careful examination is found to be due to the presence of numerous granules which do not take the stain (Figs. 30 and 34). Testing with iodine shows that the chloroplast is full of tiny starch-grains which are packed within the meshes of the reticulum of the chloroplast, either in the axis only, or in the ridges as well.

If sections of individuals having branched ridges be stained with iodine, it is seen that no starch is present, with the exception of that forming the starch-sheaths of the pyrenoids. This tends to prove that in these species the size of the axis depends largely on the condition of the cell. If little starch is present, then the axis will probably be slender, but if photosynthesis has been going on very rapidly, starch-grains begin to accumulate in such large numbers that they stretch apart the meshes of the reticulum and cause the axis to become very swollen. Later, the ridges also may be similarly distended.

Apparently the presence of large quantities of starch in the low-ridged

type gives a certain amount of rigidity to the chromatophore, and when the starch disappears the reticulum of the chloroplast collapses and the ridges become thinner but more plate-like as the axis decreases in size. In this condition they are unable to preserve their original straight paths from end to end of the chloroplast, but from lack of internal support bend irregularly all down the cell.

The distending of the chloroplast with starch-grains may occur in any species of *Closterium*, or indeed in any other Desmid, and although, so far, it has only been observed to influence the form of the chloroplast to any great extent in *Cl. costatum*, *Cl. striolatum*, *Cl. regulare*, and *Cl. angustatum*, it is quite probable that the examination of other species of the genus at various seasons of the year will show that the phenomenon is quite general with this type of chloroplast.

Slight undulation of the ridges is also sometimes observed in some of the smaller species in which the ridges are scarcely large enough to allow of real anastomosis. Thus in some individuals of *Cl. Dianae* and *Cl. rostratum* the ridges undulate in this way, whilst in others they do not. Probably this difference is also due to the same variation in the starch-content of the chromatophore.

Although the variation in the size of the axis is usually to be correlated with the amount of stroma-starch contained in it, yet in the two large species *Cl. Lunula* and *Cl. Ehrenbergii* this is not so. For in these species there is also great variation in the size of the axis of the chloroplast, the protoplasmic network of the chloroplasts of individuals having broad radiating plates and a comparatively slender axis being usually in the form of a very fine, compact reticulum, but where the axis is much broader the protoplasmic network is very coarse; cf. Figs. 2 and 4. In the latter case the appearance is not due to the presence of stroma-starch, but simply to the extreme vacuolation of the protoplasm.

In material containing a quantity of *Cl. Ehrenbergii* and a little *Cl. moniliferum*, which had been stained in the usual way, it was noticed that practically every individual of the former species contained countless numbers of small darkly staining granules, similar to those previously observed in species of *Cosmarium*. They are usually found in large numbers in the narrow ridges of the chloroplast quite near the cell-wall (Fig. 44), and never occur very far in the interior of the cell. In some individuals they are not so numerous as in others, and possibly vary in number and size according to the state of nutrition of the cell. In *Cl. moniliferum* there are sometimes similar rather smaller globules in exactly the same position in the peripheral region of the ridges. Occasionally such darkly staining globules have been observed in *Cl. Lunula*, but they were not so common in this species as in *Cl. Ehrenbergii*.

Cl. Lunula.

The ridges in this species are about fourteen to seventeen in number, and the axis in transverse section is usually broad, but the relative size of the axis and ridges is liable to considerable variation; cf. Figs. 1–4. Sometimes the ridges are so low that they scarcely exist at all in the broader part of the cell; towards the apices they are always more evident (Figs. 1 and 3). When the axis is very broad the pyrenoids are scattered in a single layer in its peripheral parts (Figs. 1 and 2). Other specimens may have very distinct ridges, which in transverse section are seen to be quite thin and plate-like, and consequently the axis in these cases is much more slender than before, the pyrenoids being necessarily crowded together in the more central region of the cell (Figs. 3 and 4). This variation in structure is quite evident even in the living condition. In certain individuals the cell-contents are much more densely green than in others. Such specimens have very distinct ridges, and the pyrenoids are crowded together in the middle of the cell, forming a large refractive mass. Other specimens are much paler in colour, and the cytoplasm is very coarsely reticulated, the reticulation being sometimes visible even with the low power. Here the pyrenoids are scattered in a more or less superficial layer, and the ridges are very low and often scarcely perceptible. Both these types of structure and various intermediate stages are to be found in a single collection. The dense green colour of the first type is probably due to the much closer reticulation of the protoplasmic foundation of the chloroplast.

In individuals having rather extensive plate-like ridges these may undulate very strongly (Fig. 3). Sometimes undulation only occurs in the apical end of the chloroplast when the ridges in the middle of the cell are too low to allow of such undulation. In transverse section, however, no branching of the ridges is to be observed, except occasionally in sections taken from the apical end of the cell, which may show just a suggestion of branching (Fig. 5). On the whole, however, the undulation in this case is merely the result of the ridges travelling in slightly waving paths, and not due to actual anastomosis, or fusion of the tissue of one ridge with that of other ridges.

The degree to which the edges of the ridges are lobed naturally depends to some extent on the size of the ridges themselves. In cells having very low ridges, the lobing of the edges may be practically absent except at the apices of the cell, where the ridges are always larger (Fig. 1). In individuals having more definite plate-like ridges the edges are usually distinctly lobed throughout their whole length, though the depth to which they are hollowed out varies (Fig. 3). Occasionally in specimens having a very slender axis with crowded pyrenoids it may be said that ridges scarcely exist, since they are represented merely by innumerable long finger-like outgrowths radiating

from the axis to the cell-wall in rather obscure longitudinal rows. Very often such a structure of the chloroplast is visible at the apices of the cell only, whilst in the broader part the ridges are more obvious.

Cl. lanceolatum and *Cl. Siliqua.*

These two species also have chloroplasts, consisting of a rather broad central axis, from the surface of which the ridges do not project very far (Figs. 6–7 and 45–6). In the external view the ridges are seen to travel in nearly parallel straight lines (Figs. 6 and 45), and although in *Cl. lanceolatum* they may undulate very slightly, they never anastomose. In the latter species the ridges are about twelve in number, whilst in *Cl. Siliqua* there are about ten or eleven. The pyrenoids lie in a single line in the middle of the cell in both cases, and in *Cl. lanceolatum* they are often provided with a very thick starch-sheath, so that when they occur in a transverse section they occupy the whole of the broad axis (Fig. 7).

Cl. costatum, *Cl. striolatum*, *Cl. regulare*, and *Cl. angustatum.*

In these species there is great variation in the relative size of the axis and ridges; in the external view, anastomosis of the ridges is consequently often seen (Figs. 31 and 38), whilst in other individuals they are nearly parallel and straight. Similarly this variation in structure is also evident when various transverse sections are examined, some sections showing a structure similar to that figured by Lutman, whilst in others the ridges may be very irregularly branched, according as they are straight or anastomose respectively (Figs. 26–30, 32–4, and 40–3). In extreme cases the axis may not have a definite rod- or cone-like form, but may merely consist of an irregular strand very much distended at the points where pyrenoids occur. *Cl. costatum* has usually thirteen or fourteen ridges, *Cl. striolatum* about thirteen, *Cl. regulare* twelve, and *Cl. angustatum* about ten. The pyrenoids occur in a single row in the middle of the cell, occasionally spreading when very numerous towards the periphery (Fig. 34). In these species the difference in appearance of the protoplasmic network of the chloroplast, and the difference in the relative size of the axis and ridges, according as much or little stroma-starch is present, is particularly noticeable.

Cl. attenuatum.

In the case of *Cl. attenuatum* the ridges are about ten in number and are seen in transverse section to be fairly large and plate-like (Figs. 11 and 12). They were never observed to anastomose, but it is quite possible that in certain circumstances they would do so. The pyrenoids are in a single line in the middle of the cell (Fig. 10).

Cl. Libellula.

This species always seems to have fairly large ridges, even when quite a considerable amount of stroma-starch is present (Fig. 9). They are about twelve in number, and are occasionally seen to anastomose, although being as a rule quite straight (Fig. 8). The pyrenoids are in a single line in the middle of the cell, and are peculiar in being usually of very great length, a single pyrenoid sometimes stretching through more than half the entire length of the chloroplast (Fig. 8). Consequently they are very few in number, about two or three in each half-cell. Excepting that a single pyrenoid rarely stretches through the whole length of the chloroplast, these pyrenoids agree in every particular with those found in the genus *Netrium* (Figs. 13–19), being provided with several layers of starch-grains. This is the only species of *Closterium* in which such pyrenoids have been observed.

In the three smaller species, *Cl. Dianae*, *Cl. rostratum*, and *Cl. juncidum*, the ridges seem to be fairly large considering the small size of the cell (Figs. 21–5, 35–7, 47, and 48). *Cl. Dianae* and *Cl. rostratum* have about five or six ridges which sometimes undulate gently (Fig. 21), whilst in the case of *Cl. juncidum* they are about seven in number.

NOTE:—The chloroplasts of *Pleurotaenium Trabecula*, (Ehrenb.) Näg., var. *rectum*, (Delp.) W. and G. S. West.

The chloroplasts in the genus *Pleurotaenium* are generally supposed to be in the form of parietal bands, with numerous pyrenoids embedded in them. In the case of *Pl. Trabecula*, var. *rectum*, it was found, however, after the examination of a large quantity of material, that the chloroplast is invariably axile, with a single row of pyrenoids in the middle of the cell (Fig. 49). A few low longitudinal ridges, which are sometimes twisted slightly in a spiral round the axis, project from its surface. There was no suggestion in any individual of the chloroplasts being even partly parietal.

V. THE CHLOROPLASTS OF THE GENUS *TETMEMORUS*.

It has long been known that the chloroplasts in *Tetmemorus* are axile, with a central row of pyrenoids. This latter character was indicated in the figures of Ralfs (1848) for the three largest species of the genus, whilst de Bary (1858) also illustrated the ridged nature of the chloroplast in *T. Brebissonii*, but since these early times no work has been done on the chloroplasts of the genus.

Three species were examined during this investigation, *T. Brebissonii*, (Menegh.) Ralfs, *T. granulatus*, (Bréb.) Ralfs, and *T. laevis*, (Kütz.) Ralfs. All these species have axile chloroplasts, but whilst those of the two smaller ones resemble each other, the chloroplast of *T. Brebissonii* is rather different.

The latter species has a central axis occupying the centre of the semi-cell, and from this about eight plates radiate towards the periphery. As these approach the cell-wall, they begin to thicken rapidly, forking slightly so as to embrace a larger proportion of the cell-wall (Fig. 81). From the exterior the edges of these plates are seen as parietal bands running longitudinally (Fig. 79). The edges of the parietal bands are often quite straight, but they may sometimes be fringed with tiny projections or else undulate gently.

The pyrenoids are confined to the central axis of the chloroplast, and are very variable in size and number. Sometimes there are only two or three long narrow pyrenoids occupying the whole length of a single chloroplast, but more often there are about four to nine smaller spherical pyrenoids, either in a single median series, or when very numerous they may occur crowded together, two or more side by side in the distended axis of the chloroplasts; cf. Fig. 80. The pyrenoids are often seen in an active state of division, the pyreno-crystal budding in various directions and being consequently very irregular in shape.

In *T. granulatus* and *T. laevis* the axile chloroplasts are also provided with a central row of pyrenoids, which number about three to five in each chloroplast of the larger species, and one or two in *T. laevis*. In both cases the axis is provided with a number of radiating plates, but in neither species is there any attempt at the formation of definite thick parietal strands as in *T. Brebissonii*. *T. granulatus* has about eight to ten radiating plates, and in *T. laevis* there are about eight, but, in the former species particularly, these are often so complicated that it is scarcely possible to count them even in sections; cf. Fig. 77.

The edges of the plates are usually deeply cut, forming long attenuated outgrowths which stretch towards the periphery, some projecting on one side of the ridge, some on the other, and when this happens the individual identity of each ridge becomes very obscure (Fig. 76). In other specimens the toothing of the edges is not so deep, and the plates are much more distinct (Fig. 78). The extent to which the ridges are toothed greatly influences the appearance of the individual. Where definite plates occur these are sometimes seen in the front view of the specimen to undulate in a manner similar to that occurring in certain species of *Closterium*, adjacent ridges alternately touching and becoming distant.

VI. The Chloroplasts of the Genus *Euastrum*.

The larger species of this genus are so very densely green in the living condition that it is impossible to get any idea of the form of their chloroplasts, even after very careful study. For this reason they have never been accurately described or figured.

The earliest investigators did not usually attempt to illustrate the

structure of the chloroplasts, and the figures of Delponte (1873) were the first to show any differentiation at all of the cell-contents. Delponte indicated the presence of ridges on the surface of the chloroplasts in *Eu. ansatum* and *Eu. oblongum*, but, on the whole, his figures do not give a very good idea of the form of the chloroplast in the genus.

Gay (1884) gives a more distinct figure of the chloroplast in *Eu. Didelta*, var. *sinuatum*, but it is incorrect, since he represents in a single semi-cell two separate chloroplasts, each with a central pyrenoid. During this work the typical chloroplast of this species was found to be quite different.

In practically all cases, careful staining is essential for the successful investigation of the chloroplasts of this genus, and with the largest species it is even necessary to cut sections in order to understand their complicated structure. After the general plan of the chloroplast has been made out by means of sections, it is then possible to interpret the extraordinary appearance presented by the whole specimen.

The species examined were *Eu. dubium*, Näg., *Eu. elegans*, (Bréb.) Kütz., *Eu. binale*, (Turp.) Ehrenb., *Eu. pectinatum*, Bréb., *Eu. ansatum*, Ralfs, *Eu. bidentatum*, Näg., *Eu. crassum*, (Bréb.) Kütz., *Eu. oblongum*, (Grev.) Ralfs, *Eu. Didelta*, (Turp.) Ralfs, *Eu. ampullaceum*, Ralfs, *Eu. affine*, Ralfs, *Eu. insigne*, Hass., *Eu. ventricosum*, Lund., *Eu. sinuosum*, Lenorm., *Eu. cuneatum*, Jenner, and *Eu. verrucosum*, Ehrenb. It was found that in every case the chloroplasts are typically axile, and in all these species, with the single exception of *Eu. verrucosum*, there is only one chloroplast in each semi-cell. It is possible that this species, which differs from all the others examined, not only in its chloroplast, but also in the general form of its cells, should be placed in a special section of the genus *Euastrum*.

Excluding *Eu. verrucosum*, the species examined fall into two distinct groups according to the structure of their chloroplasts. In the first the chloroplast is quite simple, and contains typically a single pyrenoid in the central position. The larger species form the second group. Their chloroplasts are much more complicated, and although being nominally axile, have by far their greater mass disposed in a parietal layer against the interior of the cell-wall. The pyrenoids are numerous, and are practically confined to the more peripheral parts of the chromatophore.

Although presenting very diverse appearances when examined from the exterior, the chloroplasts of the various species of the genus are really all built up on the same plan, and a comparison of transverse sections shows that the differences even between the two main groups are very slight. In fact all can be considered as elaborations of the simple type found in *Eu. dubium*, and in general the chloroplast becomes more complicated with the increase in size of the cell.

Group I.

This group comprises in general the smallest species of the genus. The chloroplast consists of a fairly massive central portion containing one or more pyrenoids, and from this radiate towards the periphery various plates which are always destitute of pyrenoids.[1] The most important part of the chloroplast in these smaller species is the central axile mass, the peripheral parts being relatively small and insignificant.

Eu. dubium, *Eu. elegans*, and *Eu. binale*.

These small species, and probably all the smallest species of the genus, have a very simple type of chloroplast. Here there is a rather massive chloroplast which occupies practically the whole of the tiny semi-cell, and consists simply of an axile mass containing typically one pyrenoid, from which four lobes are given off, two of these enveloping each front wall of the semi-cell (Figs. 56–8). Sometimes in the front view two ridges are visible in the median region, owing to the slight branching of the four primary lobes, but this is seen much better in the end view; cf. Fig. 58.

Eu. bidentatum.

Eu. bidentatum has a chloroplast which is rather different from that of any of the above-mentioned species. It sometimes has a more or less definite parietal layer of chloroplast, comparable to some extent with the parietal plates found in the larger species of the genus. The parietal portion, however, usually takes the form of irregular bands lying against the cell-wall (Fig. 51); it is only very rarely that definite parietal plates are present (Fig. 50). These bands are seen in the end view to be connected up to a central axile mass containing a single pyrenoid. They are, in fact, merely the flattened edges of the various lobes arising from the central axis (Fig. 53). There are usually four such lobes, but occasionally extra ones are present.

Eu. pectinatum and *Eu. ansatum*.

These two species, in spite of their fairly large size, have typically one pyrenoid in each semi-cell, but their chloroplasts, although being exactly of the same type as that found in the smallest species of the genus, are rather more elaborate.

There is, in these two species, a single pyrenoid embedded in a central mass of chloroplast, which occupies comparatively less space than the corresponding axis of the smaller species, whilst the ridges are in conse-

[1] In one specimen of *Eu. pectinatum* a number of small naked pyrenoids or proteid granules were observed in the peripheral parts of the chloroplasts, but in this group ordinary pyrenoids never occur except in axile part of the chromatophore.

quence much larger and more important. Four main ridges are given off from the central axis (Fig. 68), and as a rule each of these soon forks into two, sending one branch to the front wall and the other to the side wall (Fig. 61). Thus there are usually eight ridges in all, two running towards each front wall, and two towards each side wall. In the front view the chloroplast often looks more complicated owing to the extraordinary bending or further branching of these eight ridges, or the presence of extra ridges arising from the central axis (Figs. 59 and 66). The subsidiary ridges arising by the further division of the eight main ridges are seen in the front view as short dark lines. It is usually only the main ones which extend all the way from the nucleus to the apex of the semi-cell. In these two species also the edges of the ridges lying against the cell-wall are frequently lobed.

The single pyrenoid in the centre of the cell may be replaced in well-nourished specimens by a group of two to four, usually in a line, one above the other, or more rarely side by side in the central axis (Figs. 59 and 60).

The general form of the chloroplast in all these smaller species having a central pyrenoid is very similar, the chromatophore simply becoming more elaborate with the increase in size of the cell. There is very little variation amongst the individuals of each particular species, and the only deviation from the type was observed in one or two specimens of *Eu. bidentatum*, the same condition being also noticed on other occasions in *Eu. pectinatum* and *Eu. binale*. These peculiar specimens contained two pyrenoids in each semi-cell, side by side, but widely separated from each other. Moreover each pyrenoid was embedded in the centre of a separate mass of chloroplast. The semi-cell therefore contained two axile chloroplasts, each consisting of an axis containing a pyrenoid and several radiating plates stretching towards the cell-wall; cf. Figs. 54, 55, 70, and 71. It was interesting to notice this variation in the structure of the chloroplast, because in *Eu. verrucosum* (Figs. 90, 91, and 92) the chloroplast is normally of this particular type. It would be unwise, however, to attach too much importance to the coincidence, since the variation only concerned a few isolated specimens, and moreover the same kind of structure has also occasionally been observed in certain species of other genera, e.g. *Cosmarium*, which normally have a single chloroplast with one pyrenoid.

Group II.

The chloroplasts of species belonging to this group are characterized by the fact that the axile part is very thin and delicate, and contains few or no pyrenoids, whilst the cell-wall is covered with a thick parietal layer of chloroplast which is much more important and usually contains numbers of pyrenoids.

Most of the largest species examined have chloroplasts of this type, including *Eu. crassum*, *Eu. oblongum*, *Eu. Didelta*, *Eu. ampullaceum*, *Eu. affine*, *Eu. insigne*, *Eu. ventricosum*, *Eu. sinuosum*, and *Eu. cuneatum*. There is remarkable uniformity in the structure of the chloroplasts in typical specimens of all the above species, that of the last-mentioned being the only one which differs even slightly from the general type.

Although the form of the chloroplast is so very constant throughout the whole group, in each individual species there is considerable variation, the same kind of variation being met with in every case. Specimens whose chloroplasts are transitional between the axile and parietal conditions are frequently found in most of the species mentioned above.

Putting aside such exceptions, and taking the ordinary axile form of chloroplast found in *Eu. crassum* as a type, it is seen that the massive chloroplast has a central axis running through the interior of the semi-cell from nucleus to apex; cf. Fig. 100 (lower semi-cell). This gives off four radiating plates, two going towards each front face of the semi-cell (Fig. 101). On reaching the cell-wall each of these radiating plates spreads itself out against the surface of the wall, forming an extensive parietal plate, two of which can be seen in the front view of the semi-cell; cf. Fig. 100 (upper semi-cell). This parietal portion, consisting of four plates, forms by far the more massive part of the chloroplast, the central axis and radiating plates being very thin and difficult to distinguish in whole specimens.

The central axis usually has the form of a long narrow flattened plate or slender rod which is in close connexion with the nucleus near the isthmus, and often extends nearly to the median incision of the apical lobe. When more or less plate-like, it varies in its disposition, sometimes presenting its surface to the observer in the front view and sometimes its edge. Very often it is twisted at some particular point through an angle of 90°, so that for part of the way one sees its surface, and for the rest of the way its edge; cf. Figs. 63, 72, and 100. It is very common for two such twists to occur, one a little way from the nucleus and the other a similar distance from the apex.

Occasionally, especially in *Eu. Didelta*, the central axis is much broader than usual, and forms an extensive plate in the middle of the cell. In transverse sections such an axis is seen as a thin line parallel to the front walls of the cell, giving off at each end two extremely short radiating plates which spread out almost immediately to form the parietal part of the chloroplast (Fig. 65). Thus there is a concentration of chloroplast substance towards the sides of the cell, and this fact is also apparent from an examination of the whole specimen in the front view, because two dark lines are to be seen stretching from the nucleus to the apex, one on each side of the median line. In such cases it is very difficult to demonstrate the presence of the central axis of the chloroplast. It cannot as a rule be seen at all in

the front view, and it would be very easy to confuse such a chloroplast with the really parietal form which is occasionally met with in this genus. In order to determine whether the chloroplast is axile or not, it is necessary to examine it from the side or end. The central axile plate will then be viewed along its edge, and will appear as a sharp line if it be present. Such broad central axes occur occasionally in other species of the genus (cf. Figs. 82, 93, and 94), but it is the rule rather than the exception in *Eu. Didelta*.

In most specimens, pyrenoids are rarely found in the central axis, but there may be one or two small ones, especially when this part of the chloroplast is more massive than usual. If there is a twist in the axis, a tiny pyrenoid is also commonly found at the point of twisting. *Eu. sinuosum*, *Eu. ampullaceum*, and *Eu. affine*, however, often have pyrenoids even of the ordinary size in their central axis, especially in well-nourished specimens with large chloroplasts.

In transverse sections the radiating plates are seen connecting up the parietal plates with the central axis, and their length naturally depends on the shape and disposition of the latter. They may be long and distinct (Figs. 73, 99, and 101), or in rare cases they may appear to be entirely absent, the parietal part of the chloroplast apparently arising directly from the central axis; cf. *Eu. Didelta* (Fig. 65). Towards the cell-wall these radiating plates thicken out, and finally spread out over the wall to form the parietal plates (Figs. 83, 99, and 101).

The latter form a thick layer lining nearly the whole of the cell-wall, and there are four of them in each semi-cell, two side by side on each front face (Figs. 62, 63, 72, 82, 93, 94, &c.). The general shape of the parietal plate necessarily depends on the outline of the cell. Its one edge approximately follows the lateral margin of the semi-cell, and the opposite edge closely approaches the median line. The plates are sometimes coarsely lobed, and these lobes are further subdivided, forming a fringe of projections, the shape of which varies according to the species. In *Eu. oblongum* they are rounded or finger-like (Fig. 72); in *Eu. crassum* they are more jagged (Fig. 100). The lobing, particularly of those edges near the median line, is frequently very deep, and as a consequence the plates are sometimes nearly cut horizontally into two or three separate parts (Figs. 72 and 100).

The external surface of the parietal plates is rarely quite smooth, but is usually covered with tiny outgrowths extending towards the cell-wall. The size and shape of these projections vary with the species, but they are usually very similar to the teeth or outgrowths round the edges of the parietal plates of the same species. In *Eu. crassum* they are frequently very large and can be seen quite easily in living specimens (Fig. 100). In the smaller species they are not so evident, but they can usually be

distinguished in stained material, and can be recognized in transverse sections (Figs. 65, 73, 83, 97, 99, and 101). It is only occasionally, when the chloroplast is feebly developed or when the chloroplast is very distended with stroma-starch, that the parietal plates are quite smooth.

Most of the pyrenoids contained in the cell are embedded in the substance of the parietal plates. The number and size of the pyrenoids present vary considerably according to the condition of the individual cell. *Eu. crassum* and *Eu. ventricosum* seem to have the greatest number, seven or eight being scattered throughout each parietal plate, or in all about thirty to forty in each semi-cell (Figs. 82 and 100). In *Eu. Didelta*, *Eu. oblongum*, and *Eu. ampullaceum* there are about three in each parietal plate, most of them embedded in the thickest part of the chloroplast, where the radiating plates spread out to form the parietal plates (Figs. 65 and 97). *Eu. affine* has very few pyrenoids, only five to eight in each semi-cell, but these are very large (Fig. 93). *Eu. sinuosum* has about as many smaller ones (Fig. 62).

Eu. cuneatum.

Eu. cuneatum, although agreeing essentially with *Eu. crassum* in the form of its chloroplast, differs from the general type in one or two respects. The central axis is usually in the form of a broad triangular plate, its surface parallel to the front faces of the semi-cell, as is frequently the case in *Eu. Didelta*. The two lateral edges of the plate are seen in transverse section to thicken abruptly and fork into two. Thus there are four masses of chloroplast, each extending towards the cell-wall, either as a parietal plate with projections on its external surface (Figs. 84 and 87) or as a more or less brush-like mass (Fig. 89 and 85). In the latter case the whole chloroplast with the exception of the central axis is deeply cut into numerous long finger-like projections stretching towards the cell-wall, and definite parietal plates are not evident in the front view. Various stages between the comparatively smooth parietal plate and the brush-like form are to be found.

Eu. cuneatum also differs from all other members of the group in the arrangement of its pyrenoids, of which there are three to twelve in each semi-cell. When there are comparatively few pyrenoids, these are embedded in the thickened edges of the central axis, all in one plane (cf. Fig. 85), but when there are more than can be accommodated in this position they spread outwards into the parietal masses (Fig. 87). They are, however, always found in the interior of the cell rather than in the parietal parts of the chloroplast.

Occasionally *Eu. cuneatum* is found with a distinct parietal layer of chloroplast containing scattered pyrenoids, very similar to that of *Eu. crassum*, but more often it shows the structure described above.

Although the chloroplast described above is typical for all the species mentioned, it is not uncommon to find odd specimens which have a slightly different structure. The variations usually concern the axial part of the chloroplast; the parietal plates vary very little amongst individuals of the same species, the chief differences occurring in the lobing of the edges of the plate and the number and size of the outgrowths from its surface. It is quite a common thing, however, to find in many species specimens in which the central axis is much shorter than usual, leaving a space either at the apex or base of the semi-cell, or both (Figs. 104 and 105). The shape of the radiating plates is necessarily interfered with when this happens, and they accordingly curve outwards from the shortened axis towards the apex and base of the cell, so that the parietal plates are usually quite normal. In other cases both the central axis and the radiating plates may be missing, and the parietal plates are then the only remaining part of the chloroplast (Fig. 102). Sometimes the parietal plates, in such cases, are not entirely separate and free from each other, but are variously connected by one or two extremely thin strands of chloroplast running across the cell from one plate to another (Figs. 74, 75, 96, and 103).

Occasionally very irregular chloroplasts are found, in which the central axis may be perforated (Fig. 94) or displaced, together with the radiating plates, from its usual position. Another variation is seen in Figs. 86 and 88.

In typical specimens of the group, the axial part of the chloroplast, composed of such extremely thin plates, cannot play a very important part in the photosynthetic processes of the cell. This is proved to some extent by the usual absence of pyrenoids from the central axis in the largest species of the genus. The majority are always to be found in the parietal plates. Considering this fact, and also that parietal chloroplasts have been evolved in a few advanced species of other genera of Desmidiaceae, it might be expected that in these large species of *Euastrum* there would be a tendency for the useless part of the chloroplast in the interior of the cell to disappear, leaving just the parietal plates. This is possibly the reason why so many individuals seem to show transitional stages between the axile and parietal condition.

A less important variation was observed in two isolated specimens, one of *Eu. crassum*, and the other of *Eu. oblongum*. Here all the pyrenoids were aggregated in a compact irregular mass in the interior of the cell. The central axis was considerably swollen to accommodate them, and from it a number of short strands of chloroplast ran out towards the cell-wall, ending, in the first-mentioned species, in irregular parietal bands. In the specimen of *Eu. oblongum* there was a more or less complete reticulated layer of chloroplast lining the cell-wall. These two specimens were interesting because they appear to form a link between Groups I and II, and this

was all the more striking because an unusual specimen of *Eu. ansatum* (Group I) was also encountered which was almost identical with the abnormal specimen of *Eu. crassum* described above.

The Chloroplasts of *Eu. verrucosum*.

This species differs from all the other species examined in having two chloroplasts in each semi-cell. In each chloroplast there is typically one large pyrenoid, and the general appearance of the cell is very suggestive of certain large species of *Cosmarium*. Each chloroplast is provided with an axis, which, beginning at the isthmus, stretches diagonally towards the incision between the apical and lateral lobes; cf. Fig. 91. This axis contains a single large pyrenoid, and around it are arranged about four or five irregular ridges which radiate towards the front and side walls of the cell (Figs. 90 and 92). The peripheral edges of the ridges are usually very little ornamented.

Thus the chloroplast of *Eu. verrucosum* is very unlike that of any other species examined, and it is therefore very difficult to explain its relationships. It is possible, however, that the examination of other more closely allied species would help to make clear its affinities.

VII. The Chloroplasts of the Genus *Xanthidium*.

In a few species of the genus, the form of the chloroplast can be made out from living material, and the chloroplasts of one or two species were figured by Delponte (1873). The structure of the chloroplast and the position of the pyrenoids were also indicated in several species by W. and G. S. West (1904–11).

Boldt (1888) attempted to subdivide the genus according to the disposition of the chloroplasts, and instituted the sub-genera *Euxanthidium* and *Centrenterium* to include those species having parietal and axile chloroplasts respectively.

Larsen (1907) described and figured *X. groenlandicum*, Boldt, indicating that in its chloroplasts this species agrees fairly well with certain species of the genus having a distinctly parietal chloroplast, whereas Boldt placed it in *Centrenterium*.

During this investigation the following species were examined:—*X. aculeatum*, Ehrenb., *X. acanthophorum*, Nordst., *X. hastiferum*, W. B. Turn., *X. Brebissonii*, Ralfs, *X. antilopaeum*, (Bréb.) Kütz., *X. cristatum*, Bréb., *X. subhastiferum*, var. *Murrayi*, W. and G. S. West, *X. fasciculatum*, Ehrenb., and *X. armatum*, (Bréb.) Rabenh. The chloroplasts of these species were found to be of two distinct types, either axile or parietal, but in at least one species the form of the chloroplast is variable, and transitions from the axile to the parietal condition were observed.

X. aculeatum, *X. acanthophorum*, and *X. hastiferum*.

These three species were found to have axile chloroplasts. In all cases there are two chloroplasts in each half-cell (Figs. 117, 118, and 127). Each chloroplast is provided with an axis which arises on one side of the isthmus near the nucleus and extends obliquely towards the corresponding lateral region of the semi-cell, increasing in size and containing typically one large pyrenoid; cf. Fig. 127 (lower semi-cell).

Around this axis are arranged a varying number of plates which radiate towards the cell-wall in various directions. Four of these plates are usually more prominent than the others, one extending to each front face of the semi-cell, and two to the corresponding lateral face (Fig. 128). Other plates or ridges, if present, are often much more irregular, and do not extend from end to end of the semi-cell in the same way as the four larger ones (Figs. 117 and 118). The margins of the plates may be quite smooth, irregular, or lobed.

X. Brebissonii.

In this species the chloroplasts in typical specimens are exactly similar to those of *X. aculeatum*, *X. hastiferum*, and *X. acanthophorum*, there being in each semi-cell two axile chloroplasts each with a single pyrenoid (Figs. 119, 120, and 121). In many cases, however, the pyrenoids are much more numerous, as many as nine being present in one semi-cell, and the form of the chloroplasts may be considerably changed. Very often there are two or three pyrenoids in the axis of each chloroplast where typically there should be but one (Figs. 122 and 123), or again there may be two or three very small pyrenoids in the peripheral edges of one or more of the plates radiating from the axis (Fig. 123). The actual form of the chloroplast in such cases is often not seriously interfered with, and there are as before two axile chloroplasts in each semi-cell. In other individuals the numerous pyrenoids are not confined to the centre of the chloroplast, but, on the other hand, pyrenoids of considerable size may also occur in the more peripheral parts of the cell, showing the increased importance of the parietal parts of the chloroplast even although as a whole it may still be axile (Fig. 124). In other specimens the pyrenoids are quite absent from the positions in the interior of the cell in which they should typically occur, and the form of the chloroplast is quite different, being practically parietal, often very irregular in shape, consisting of a variable number of masses, each of which contains several scattered pyrenoids (Fig. 125). Occasionally there are four definite parietal plates each with a pyrenoid, regularly arranged, as in those species of the genus which typically have parietal chloroplasts (Fig. 126).

The axile condition of the chloroplast seems to be far more common than the parietal, and of sixteen specimens examined only five exhibited

any marked tendency to the parietal condition, and even in these one semi-cell in every case had distinctly axile chloroplasts. Sometimes in the same semi-cell one chloroplast was distinctly axile, whilst in the other half of the semi-cell there were signs of the tendency to the parietal condition.

X. antilopaeum, *X. cristatum*, *X. subhastiferum*, var. *Murrayi*,[1] *X. fasciculatum*, and *X. armatum*.

These remaining five species all have parietal chloroplasts, of which there are typically four in each semi-cell, two side by side on each front face (Figs. 106, 108, 113, 115, and 129). The parietal plates are usually very thin, and adhere closely to the cell-wall, corresponding fairly accurately with it in shape. In *X. armatum* the chloroplast plates are often prolonged at the angles of the cell to form projections which fill up the hollow bases of the branched spines (Figs. 129, 131, and 132). Very often there is an extra chloroplast on one or both front faces of the cell, either a small one, wedged in between the others, or one equal to them in size (Fig. 109). Rarely there is a fairly large number of small rounded plates, seven such chloroplasts having been observed on one front face of a semi-cell of *X. subhastiferum*, var. *Murrayi*. Occasionally in *X. armatum* the chloroplast is in the form of a number of rather narrow parietal ribbons, running longitudinally, about six to eight in each semi-cell.

The edges and external surface of the chloroplast plates in *X. subhastiferum*, var. *Murrayi*, and *X. cristatum*, appear to be quite smooth and without projections of any kind; cf. Figs. 108, 109, and 115. The same is usually the case in *X. antilopaeum* (Fig. 106), although it was occasionally noticed that in this species the surface of the chloroplasts was more irregular. In *X. fasciculatum*, however, the edges and surface of the plates were almost invariably covered with numerous short outgrowths (Fig. 113), and it was in this species that the maximum development of the chloroplast in this direction was noticed. In *X. armatum* the presence of such projections is a very variable character, being dependent on the amount of stroma-starch contained in the chloroplast. When little starch is present in the chloroplast plates are very thin, their protoplasmic foundation is dense in texture, and in such cases a few slight prominent ridges (Fig. 130) or more numerous delicate projections usually stretch from their external surface towards the periphery. When more starch is present, the chloroplasts become distended to three or more times their former thickness, and here the external surface is usually quite smooth, the delicate ridges having been quite obliterated in the process (Figs. 129 and 131).

[1] This Desmid was erroneously figured by W. and G. S. West (1904–11, vol. iv) as having an axile chloroplast with a single pyrenoid in each semi-cell.

In practically all cases each parietal chloroplast is in close connexion with the nucleus at the base of the semi-cell; cf. Fig. 106.

With the exception of the large species *X. armatum*, there is typically one pyrenoid in the centre of each parietal chloroplast (Figs. 106, 109, 113, and 115), although very often two or three may be seen close together in one or more of the plates (Fig. 108). The pyrenoids are often very large, and the thin chloroplasts are in consequence thickened considerably where they occur, the corresponding chloroplasts of the two front faces of the semi-cell sometimes projecting so far into the interior of the cell that they nearly touch at these points; cf. Figs. 110 and 111. In *X. armatum* the pyrenoids are more numerous, numbering about five to twelve in each chloroplast (Figs. 129–32). They vary considerably in size, some of them being extremely small, and comparable to the small globules found in the parietal parts of the chloroplast in *Cosmarium ochthodes* and other species.

VIII. Summary of the General Characters of the Chloroplasts.

The chloroplasts in the Saccodermae are, with a few exceptions, usually very simple in form, and are nearly all well known.

In the higher Desmids the chloroplasts are, in general, very complicated and beautiful structures, and many of them have not hitherto been investigated. The delicacy of their form is dependent upon—

(*a*) the relative amount of chlorophyll-bearing substance present in the individual, and

(*b*) the general physiological condition of the cell, particularly as regards the quantity of free stroma-starch present in the chromatophore.

The chloroplasts may be axile or parietal, and their general form is usually constant, but in a few cases there is marked variation in the disposition of the chloroplasts amongst individuals of one species.

The number and position of the pyrenoids present depend on the size and shape of the chromatophore. In the more extensive chloroplast plates of both axile and parietal forms they are often very numerous and scattered.

In all cases the amount of food stored in the form of pyrenoids is dependent on the condition of the cell, and at any time two or more pyrenoids may be formed by the division of an original one.

In many forms the number of pyrenoids present in each semi-cell is generally supposed to be constant. This is not true, since the actual number of pyrenoids present is dependent on various changing factors, but in such cases the points of pyrenoid formation are usually quite definite and fixed.

In addition to the ordinary large pyrenoids which are usually provided

with a starch-sheath, in several species numbers of small naked pyrenoids or granules of protein were observed in the superficial layers of the chromatophore.

A. *Summary of the Special Characters of* NETRIUM *and* CLOSTERIUM.

The chloroplast of *Netrium* consists of seven to twelve plates radiating from a central axis.

The central axis of each chloroplast is occupied by a single long pyrenoid stretching throughout its whole length and surrounded by a thick sheath of starch which is almost invariably more than one layer of grains in thickness.

Under certain conditions the pyreno-crystal of this long pyrenoid breaks up into numerous spherical or irregularly shaped portions which remain embedded in the central mass of starch grains.

The only other genus in which these peculiar pyrenoids have been observed to occur extensively is *Penium*, although they are also general in the two species *Cylindrocystis Brebissonii* and *Closterium Libellula*.

Lutman's statement that the chloroplast in *Closterium* consists of a curved cone-shaped structure with relatively low ridges on its surface is not true for all species of this genus.

The relative size of the axis and ridges varies with the species, and also to some extent within each individual species, according to the physiological condition of the cell.

The pronounced undulation or anastomosis of the ridges frequently seen in certain species of the genus only occurs in individuals having fairly large ridges. Such individuals show a very irregularly branched structure in transverse section and are nearly always destitute of stroma-starch.

Other individuals of these particular species containing much stroma-starch usually have a much broader axis and comparatively low ridges, which are seen in the front view to be practically parallel.

Even in species such as *Cl. Lunula* and *Cl. Ehrenbergii*, where the pyrenoids are arranged in the peripheral parts of the axis, there is considerable variation in the relative size of the axis and ridges, often to be correlated with the variation in texture of the protoplasmic reticulum of the chloroplast, and therefore the relative position of the pyrenoids is also subject to a certain amount of variation.

In spite of the fact that all species of *Pleurotaenium* are supposed to have parietal chloroplasts, it was observed that in *Pl. Trabecula*, var. *rectum*, the chloroplast consists of an axile rod, containing pyrenoids, with several low ridges projecting from its surface.

B. *Summary of the Special Characters of* TETMEMORUS.

The chloroplast in *Tetmemorus* is essentially similar to that of *Closterium* in consisting of a central axis containing pyrenoids and a number of radiating ridges.

In *T. Brébissonii* the ridges thicken abruptly towards the periphery, forming longitudinal parietal bands of considerable width.

T. granulatus and *T. laevis* have simple ridges which do not form such extensive parietal masses, but end with toothed edges, or are provided with attenuated projections which stretch towards the cell-wall. When little or no stroma-starch is present in the chloroplasts of these two species, the ridges often undulate in a manner similar to that occurring in certain species of *Closterium*.

C. *Summary of the Special Characters of* EUASTRUM.

With the exception of *Eu. verrucosum* the chloroplasts of all the species examined can be considered as variations of one type. At the same time they naturally form two distinct groups.

The smallest species examined form Group I. They have a relatively large axile mass of chloroplast containing typically one pyrenoid, and from this four main lobes project towards the periphery. These lobes are in the very smallest species quite small and insignificant, but in the larger species of the group they are somewhat larger and may be branched.

Group II contains most of the largest species examined. Here the structure of the chloroplast is essentially similar to that of the first group, but the real axis of the chloroplast is very thin, and the peripheral lobes spread out to form a thick layer lining the cell-wall, so that the greater mass of the chloroplast is in the parietal position. The pyrenoids, instead of occurring in the centre of the semi-cell as in the first group, are to be found in the more massive part of the chloroplast forming the parietal plates, the interior of the cell being often quite free from them. Following on the decrease in importance of the axile part of the chloroplast in Group II is a decided tendency for it to disappear altogether, leaving only the parietal mass of chloroplast with its pyrenoids lining the cell-wall. Transitions from the axile to the parietal condition of the chloroplast were observed in most of the larger species examined.

In the case of *Eu. verrucosum* there are two distinct axile chloroplasts in each semi-cell, each with a large central pyrenoid, as in many species of *Cosmarium*. The structure of its chloroplast suggests that it has no very close affinities with any of the other species of the genus examined, but if other related species could be investigated, its extraordinary structure would possibly be explained.

D. *Summary of the Special Characters of* XANTHIDIUM.

In those species of *Xanthidium* examined, two distinct types of chloroplast were observed.

A few species were found to have axile chloroplasts. These invariably had two chloroplasts in each semi-cell, there being typically one pyrenoid in the centre of each.

The remaining species examined had parietal chloroplasts, and here there were usually four in each semi-cell. In all these species, with the exception of *X. armatum*, there was typically one pyrenoid in the centre of each chloroplast, but in this very large species the pyrenoids were both numerous and scattered.

X. Brebissonii seems usually to have chloroplasts of the first type, but the pyrenoids frequently become very numerous, and the chloroplasts often tend to become partly parietal.

In conclusion, I wish to express my sincerest thanks to Professor G. S. West for much valuable help and advice during the course of the work, and also for supplying much of the material. I am also deeply indebted to the Birmingham Natural History and Philosophical Society for a grant from their Endowment of Research Fund, to help in the cost of reproducing the plates, and also to the Royal Society for a further grant for the same purpose.

THE BOTANICAL LABORATORY,
UNIVERSITY OF BIRMINGHAM.

BIBLIOGRAPHY.

DE BARY, A. (1858): Untersuchungen über die Familie der Conjugaten. Leipzig, 1858.

BOLDT, R. (1888): Desmidieer från Grönland. Bihang till k. svenska vet.-akad. handlingar, Band xiii, No. 5, 1888.

DELPONTE, J. B. (1873): Specimen Desmidiacearum subalpinum. Augustae Taurinorum, 1873, Mem. d. R. Accad. d. Scienze di Torino, ser. 2, xxviii.

DUCELLIER, F. (1917): Notes sur le pyrénoide dans le genre *Cosmarium* Corda. Bull. de la Soc. Bot. de Genève, 2me série, vol. ix, 1917.

GAY, F. (1884): Essai d'une Monographie locale des Conjuguées. Montpellier, 1884.

KLEBS, G. (1879): Ueber die Formen einiger Gattungen der Desmidiaceen Ostpreussens. Schriften d. Physik. Oek. Gesellsch. zu Königsberg, 1879.

LAGERHEIM, G. (1888): Ueber Desmidiaceen aus Bengalen nebst Bemerkungen über die geographische Verbreitung der Desmidiaceen in Asien. Bihang till K. Svenska vet.-akad. Handlingar, Band xiii, No. 9, 1888.

LARSEN, E. (1907): Ferskvandsalger fra Vest-Grønland. Arbejder fra den Botaniske Have i København, No. 36, 1907.

LUNDELL, P. M. (1871): De Desmidiacies quae in Suecia inventae sunt, observationes criticae. Nova Acta R. S. S. Upsala, ser. 3, xiii, 1871.

LÜTKEMÜLLER, J. (1893): Beobachtungen über die Chlorophyllkörper einiger Desmidiaceen. Oesterr. bot. Zeitschrift, 1893, Nr. 1 u. 2.
——————— (1895): Ueber die Gattung *Spirotaenia*, Bréb. Ibid., 1895, Nr. 1 u. ff.
——————— (1902): Die Zellmembran der Desmidiaceen. Beiträge zur Biologie der Pflanzen, Band viii, 1902.
——————— (1903): Ueber die Gattung *Spirotaenia*, Bréb. Oesterr. bot. Zeitschrift, 1903.
LUTMAN, B. F. (1910): The Cell Structure of *Closterium Ehrenbergii* and *Closterium moniliferum*. Bot. Gazette, xlix, 1910.
——————— (1911): Cell and Nuclear Division in *Closterium*. Bot. Gazette, li, 1911.
NÄGELI, C. (1849): Gattungen einzelliger Algen. Zürich, 1849.
RALFS, J. (1848): The British Desmidiaceae. London, 1848.
SCHMITZ, FR. (1882): Die Chromatophoren der Algen. Bonn, 1882.
WEST, G. S. (1904): The British Freshwater Algae. Cambridge, 1904.
——————— (1914): A Contribution to our Knowledge of the Freshwater Algae of Columbia. Mém. de la Soc. neuchâteloise des Sci. Nat., 1914.
WEST, W. and G. S. (1896): On some New and Interesting Freshwater Algae. Journ. Roy. Micro. Soc., 1896.
——————— (1897): Desmids from the United States. Journ. Linn. Soc., vol. xxxiii, 1897.
——————— (1898): Observations on the Conjugatae. Ann. Bot., vol. xii, 1898.
——————— (1902): A Contribution to the Freshwater Algae of the North of Ireland. Trans. Roy. Irish Acad., 1902.
——————— (1903): Notes on Freshwater Algae. Journ. Bot., 1903.
——————— (1904–11): Monograph of the British Desmidiaceae. Ray Soc., vol. i, 1904; vol. ii, 1905; vol. iii, 1908; vol. iv, 1911.
——————— (1907): Freshwater Algae from Burma. Ann. Roy. Bot. Gardens, Calcutta, 1907.

DESCRIPTION OF PLATES XIV–XVIII.

Illustrating Miss Nellie Carter's paper on the Chloroplasts of Desmids.

The specific characters of Desmids are sometimes obliterated during the prolonged processes of preparation, but all the species were identified in either the living or carefully fixed condition by Professor G. S. West.

PLATE XIV.

Figs. 1–5. *Closterium Lunula*, (Müll.) Nitzsch. Fig. 1 × 350; Fig. 2, transverse section of a similar specimen, × 810; Fig. 3, another individual, the ridges of whose chloroplast are much broader, × 350; Fig. 4, transverse section of a similar specimen, × 810; Fig. 5, transverse section near apex of cell, × 810.

Figs. 6, 7. *Cl. lanceolatum*, Kütz. Fig. 6 × 510; Fig. 7, transverse section (intervening cytoplasm more deeply stained than the chloroplast itself), × 810.

Figs. 8, 9. *Cl. Libellula*, Focke. Fig. 8 × 510; Fig. 9, transverse section (axis distended with starch), × 810.

Figs. 10–12. *Cl. attenuatum*, Ehrenb. Fig. 10 × 510; Fig. 11, transverse section near apex of cell, × 810; Fig. 12, transverse section from thicker part, × 810.

Figs. 13–15. *Netrium Digitus*, (Ehrenb.) Itzigs. and Rothe. Fig. 13, whole specimen, × 510; Fig. 14, longitudinal median section, × 510; Fig. 15, transverse section, × 510.

Figs. 16–18. *N. oblongum*, (de Bary) Lütkem., var. *cylindricum*, W. and G. S. West. Fig. 16, specimen having one long pyrenoid in each chloroplast, × 510; Fig. 17, specimen having many small pyrenoids, × 510; Fig. 18, optical section, × 510.

Fig. 19. *N. interruptum*, (Bréb.) Lütkem. Individual having one pyrenoid and one chloroplast which is just beginning to constrict, probably a recently divided specimen. × 510.

Fig. 20. *Penium spirostriolatum*, Barker. Transverse section. × 510.

PLATE XV.

Fig. 21–5. *Closterium rostratum*, Ehrenb. Fig. 21, specimen whose ridges undulate slightly, × 510; Fig. 22, another specimen with straight ridges, × 510; Fig. 23, longitudinal section, × 510; Fig. 24, transverse section, × 810; Fig. 25, transverse section near apical end of chloroplast, × 810.

Figs. 26, 27. *Cl. angustatum*, Kütz. Successive transverse sections. × 810.

Figs. 28–30. *Cl. regulare*, Bréb. Transverse sections of different individuals. × 810.

Figs. 31–4. *Cl. costatum*, Corda. Fig. 31 × 510; Figs. 32–4, transverse sections of different individuals, × 810.

Figs. 35–7. *Cl. Dianae*, Ehrenb. Fig. 35 × 510; Figs. 36 and 37, transverse sections, × 810.

Figs. 38–43. *Cl. striolatum*, Ehrenb. Fig. 38 × 510; Fig. 39, part of a longitudinal tangential section showing anastomosis of the ridges, × 810; Fig. 40, transverse section showing the distending of the chloroplast with stroma-starch; Figs. 41–3, serial transverse sections of another individual containing little or no stroma-starch, and showing the varying combinations of the ridges, × 810.

Fig. 44. *Cl. Ehrenbergii*, Menegh. Surface view showing proteid granules. × 810.

Figs. 45, 46. *Cl. Siliqua*, W. and G. S. West. Fig. 45 × 510; Fig. 46, transverse section, × 810.

Figs. 47, 48. *Cl. juncidum*, Ralfs. Fig. 47 × 510; Fig. 48, transverse section, × 810.

Fig. 49. *Pleurotaenium Trabecula*, (Ehrenb.) Näg., var. *rectum*, (Delp.) W. and G. S. West × 510.

PLATE XVI.

(All × 510 with the exception of Figs. 77 and 81).

Figs. 50–5. *Euastrum bidentatum*, Näg. Figs. 50 and 51, front view; Fig. 52, side view; Fig. 53, end view; Fig. 54, an unusual specimen having two chloroplasts and two pyrenoids in each semi-cell; Fig. 55, end view of same specimen.

Figs. 56–8. *Eu. dubium*, Näg. Fig. 56, front view; Fig. 57, side view; Fig. 58, end view.

Figs. 59–61. *Eu. ansatum*, Ralfs. Fig. 59, front view; Fig. 60, side view; Fig. 61, transverse section.

Fig. 62. *Eu. sinuosum*, Lenorm. Front view.

Figs. 63–5. *Eu. Didelta*, (Turp.) Ralfs. Fig. 63, front view, lower semi-cell in optical section; Fig. 64, longitudinal median section, showing the central axis of each semi-cell and the radiating plates cut irregularly; Fig. 65, transverse section.

Figs. 66–71. *Eu. pectinatum*, Bréb. Fig. 66, front view; Figs. 67 and 68, transverse sections; Fig. 69, side view; Fig. 70, an unusual specimen; Fig. 71, upper semi-cell of the same individual in optical transverse section.

Figs. 72–5. *Eu. oblongum*, (Grev.) Ralfs. Fig. 72, front view, lower semi-cell in optical section; Fig. 73, typical transverse section; Figs. 74 and 75, transverse sections of unusual specimens.

Figs. 76, 77. *Tetmemorus granulatus*, (Bréb.) Ralfs. Fig. 76, slightly oblique front view; Fig. 77, transverse section, × 810.

Fig. 78. *Tet. laevis*, (Kütz.) Ralfs. Front view.

Figs. 79–81. *Tet. Brébissonii*, (Menegh.) Ralfs. Fig. 79, oblique front view; Fig. 80, longitudinal median section, passing through the entire ridge on the right, and through the parietal expansion only on the left; Fig. 81, transverse section, × 810.

PLATE XVII.

(All × 510.)

Figs. 82, 83. *Eu. ventricosum*, Lund. Fig. 82, front view; Fig. 83, transverse section.

Figs. 84–9. *Eu. cuneatum*, Jenner. Figs. 84 and 89, front view; Figs. 85 and 87, transverse sections; Fig. 88, unusual specimen with part of one of its parietal plates free from the rest of the chloroplast; Fig. 86, optical transverse section of the same individual in the upper region of the semi-cell.

Figs. 90–2. *Eu. verrucosum*, Ehrenb. Fig. 90, front view; Fig. 91, longitudinal section; Fig. 92, transverse section (upper chloroplast rather torn).

Fig. 93. *Eu. affine*, Ralfs. Front view.

Figs. 94–7. *Eu. ampullaceum*, Ralfs. Fig. 94, front view of an individual with imperfectly developed chloroplast, possibly after rapid cell-division; Fig. 95, typical front view; Fig. 96, transverse section of specimen with entirely parietal chloroplasts; Fig. 97, transverse section of typical specimen.

Figs. 98, 99. *Eu. insigne*, Hass. Fig. 98, front view; Fig. 99, transverse section.

Figs. 100–5. *Eu. crassum*, (Bréb.) Kütz. Fig. 100, front view, lower semi-cell in optical section; Fig. 101, transverse section; Fig. 102, specimen having an entirely parietal chloroplast; Fig. 103, optical longitudinal section of specimen having a chloroplast which is practically parietal, but has two strands passing through the interior of the cell; Figs. 104 and 105, optical longitudinal sections showing the shortening of the central axis.

PLATE XVIII.

Figs. 106, 107. *Xanthidium antilopaeum*, (Bréb.) Kütz. × 510. Fig. 106, front view; Fig. 107, side view.

Figs. 108–12. *X. subhastiferum*, West, var. *Murrayi*, W. and G. S. West. × 510. Figs. 108 and 109, front view; Fig. 110, longitudinal section passing through pyrenoids in the lateral region of the cell (contents of lower semi-cell not shown); Fig. 111, longitudinal section nearer the middle line; Fig. 112, transverse section.

Figs. 113, 114. *X. fasciculatum*, Ehrenb. × 510. Fig. 113, front view; Fig. 114, end view.

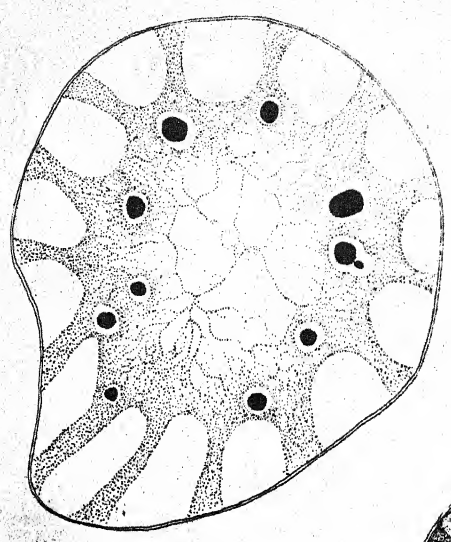
Figs. 115, 116. *X. cristatum*, Bréb. × 510. Fig. 115, front view; Fig. 116, end view.

Figs. 117, 118. *X. aculeatum*, Ehrenb. × 510. Fig. 117, front view; Fig. 118, optical transverse section.

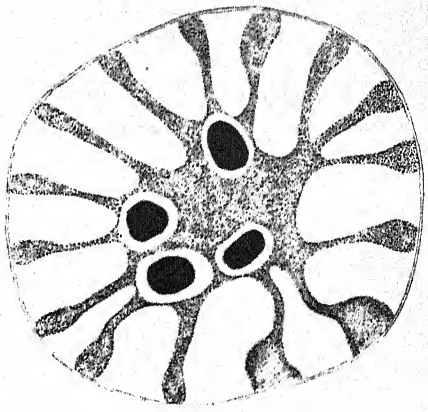
Figs. 119–26. *X. Brebissonii*, Ralfs. × 510. Figs. 119, 120, and 121, front view, side view and optical transverse section of a typical specimen; Figs. 122–6, optical transverse sections of various individuals, showing the variation in number and size of the pyrenoids, and transitions from the axile to the parietal disposition of the chloroplasts.

Figs. 127, 128. *X. acanthophorum*, Nordst. × 810. Fig. 127, front view, lower semi-cell in optical section; Fig. 128, side view.

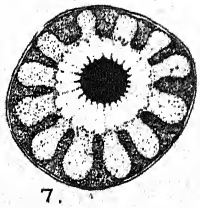
Figs. 129–32. *X. armatum*, (Bréb.) Rabenh. × 510. Fig. 129, front view of individual whose chloroplast is packed with stroma-starch; Fig. 130, front view of specimen containing little starch except that round the pyrenoids; Fig. 131, longitudinal section; Fig. 132, transverse section.



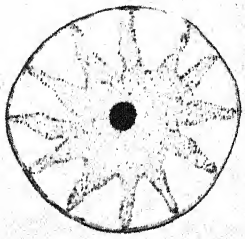
2.



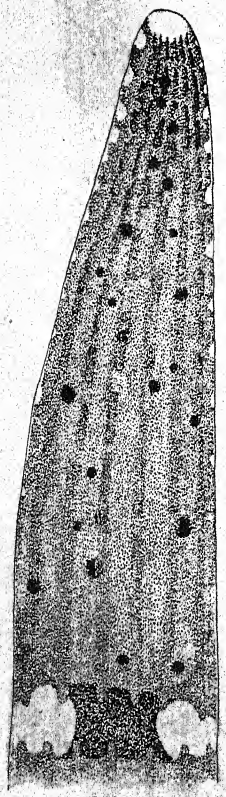
4.



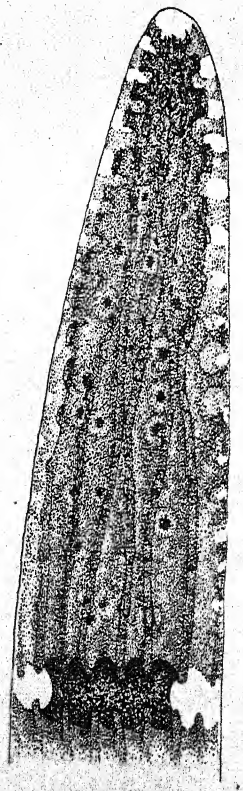
7.



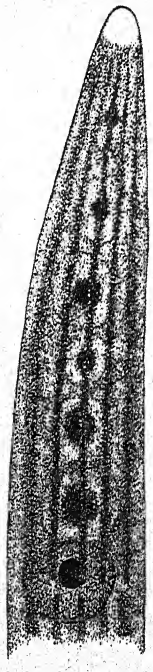
9.



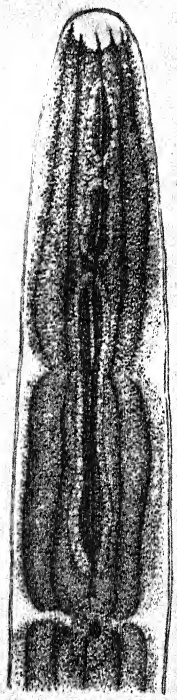
1.



3.

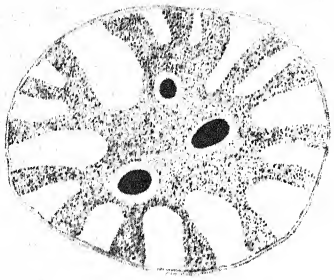


6.

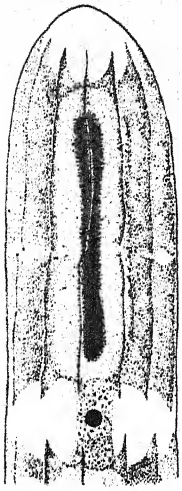


8.

CARTER.N.—CHLOROPLASTS OF DESMIDS.



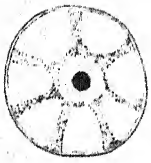
5.



19.

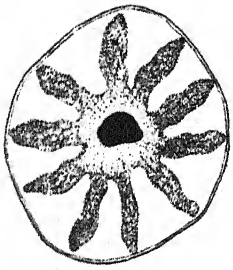
17.

16.

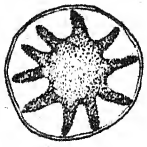


20.

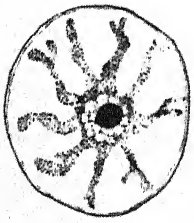
18.



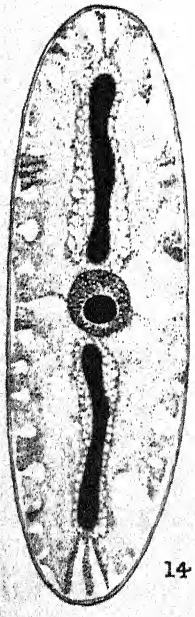
12.



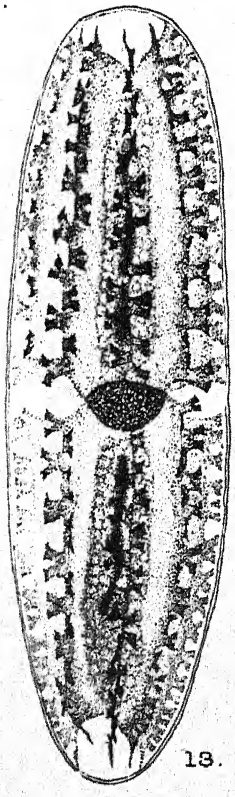
11.



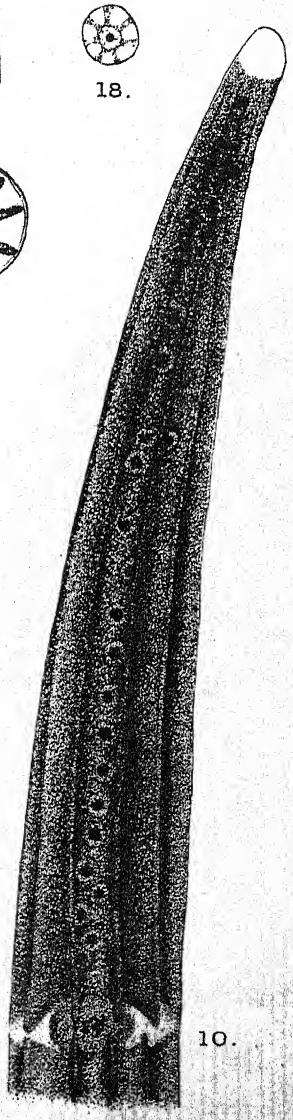
15.



14.

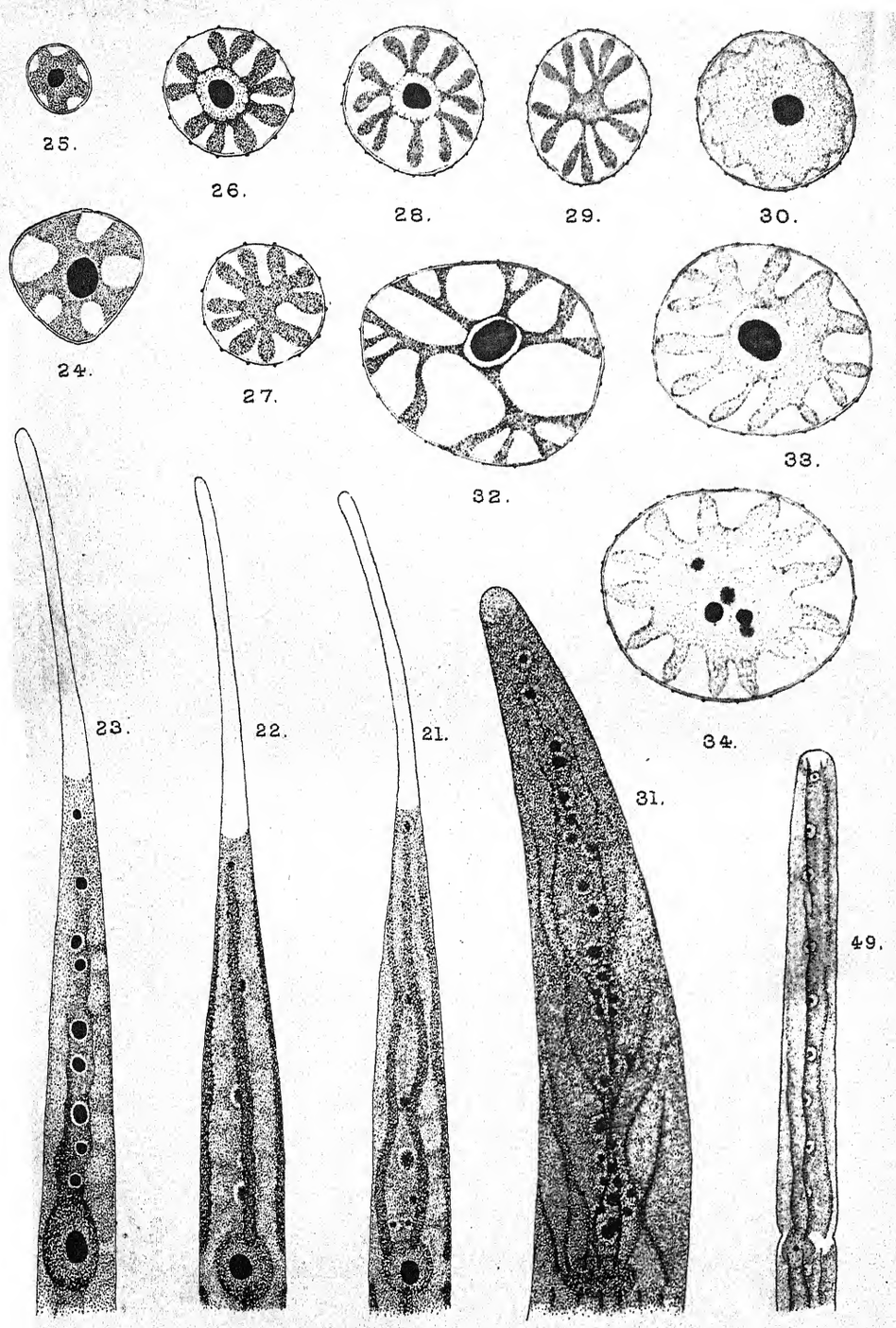


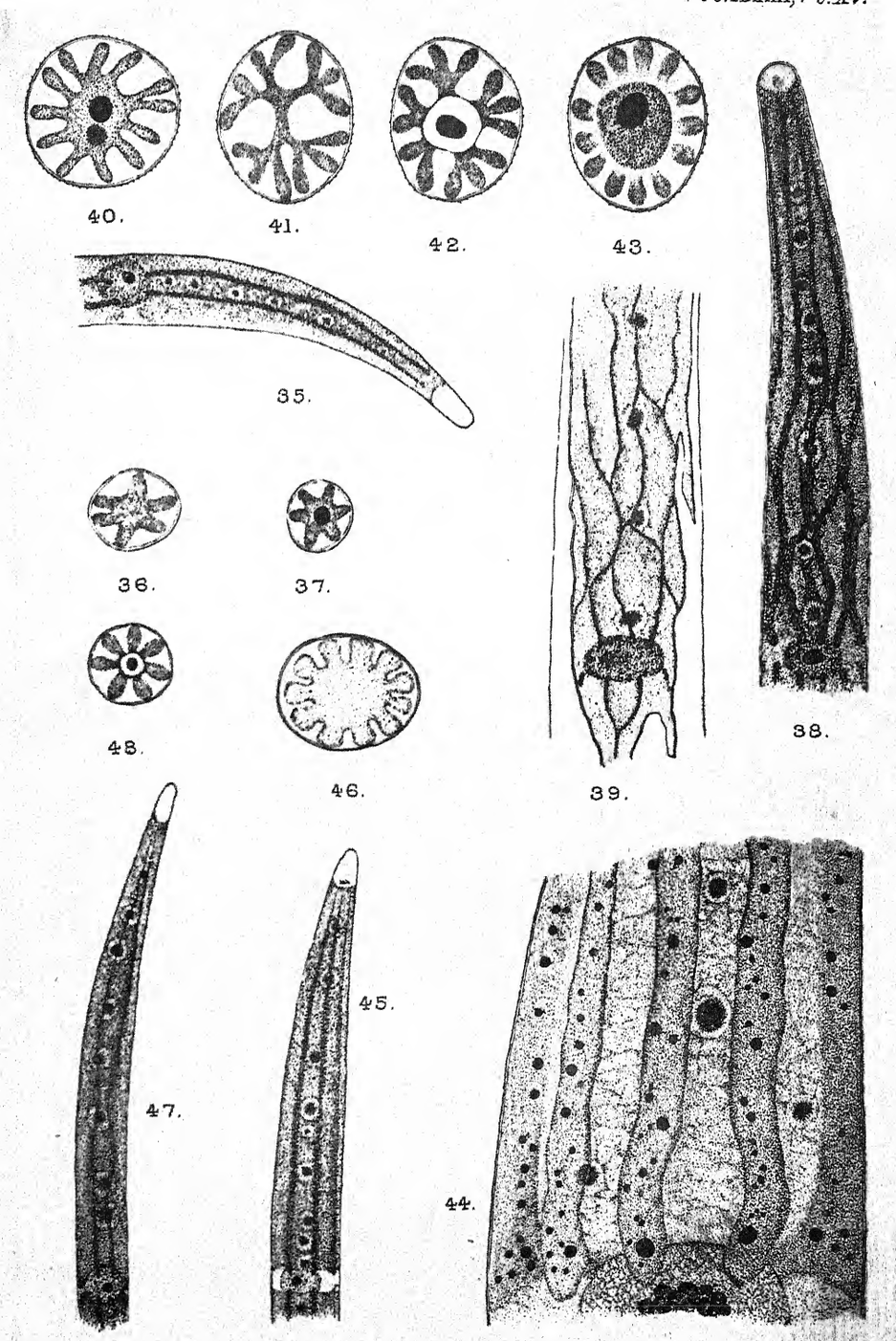
13.



10.

Huth, London.





CARTER.N. CHLOROPLASTS OF DESMIDS.

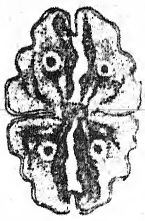
Huth, London.

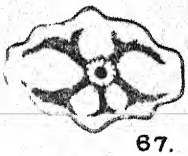
51.

52.

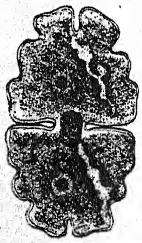
55.

53.


54.


67.

68.

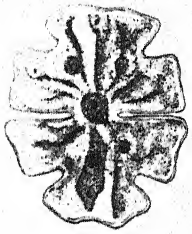

50.

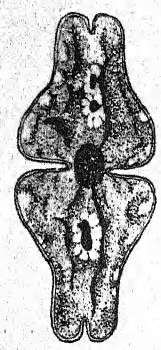
56.

57.

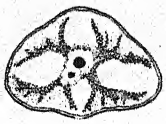
58.

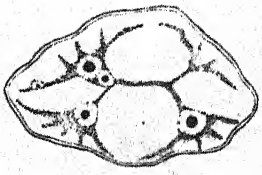
71.

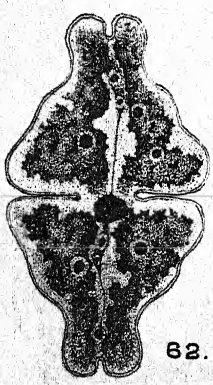

70.

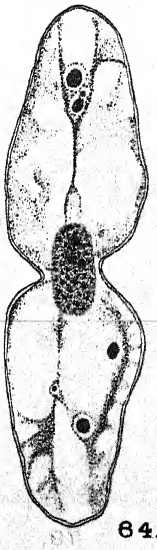

59.

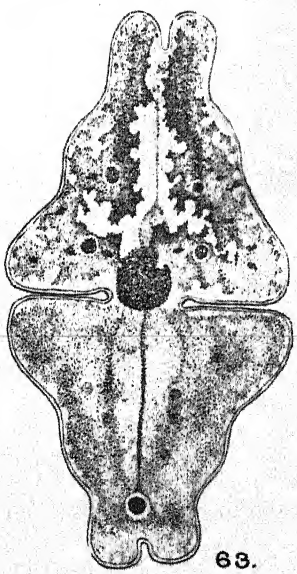
60.


61.

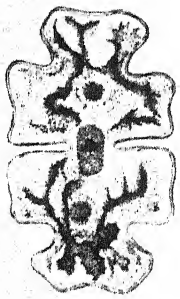

65.


62.


64.

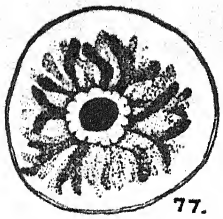

63.

CARTER.N. CHLOROPLASTS OF DESMIDS.

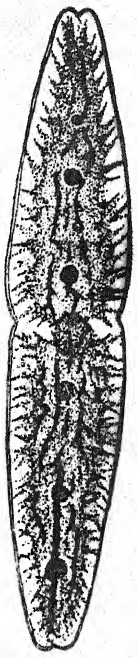


66.

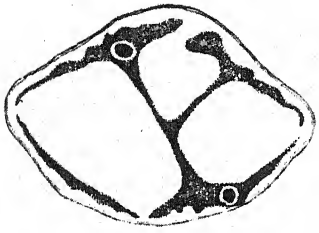
78.



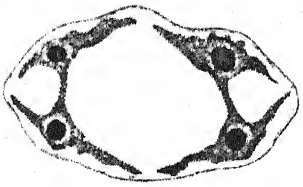
77.



76.

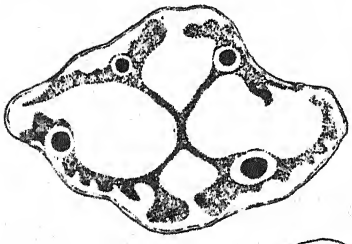


75.

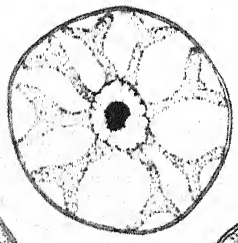


74.

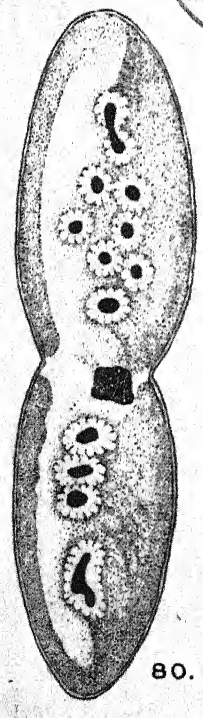
69.



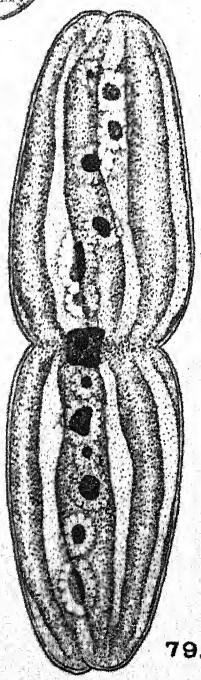
73.



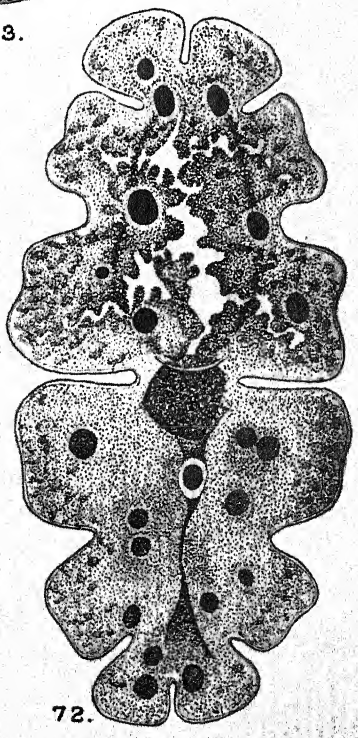
81.



80.

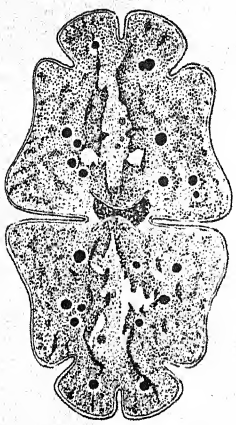


79.

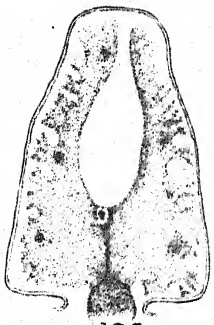


72.

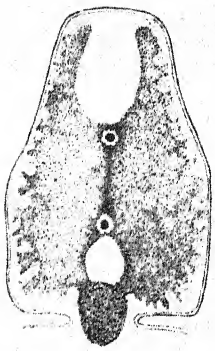
Huth, London.



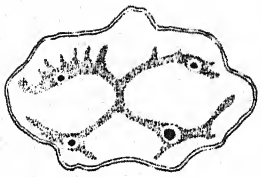
82.



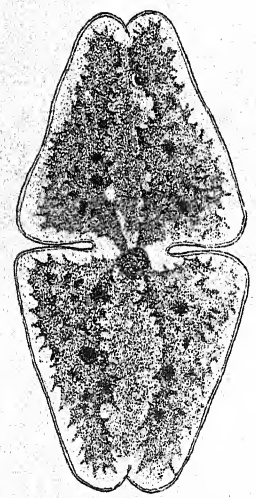
105.



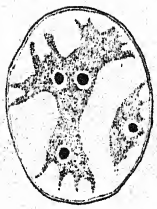
104.



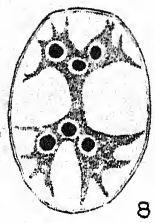
83.



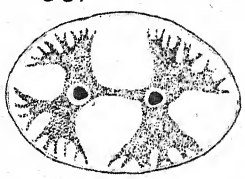
84.



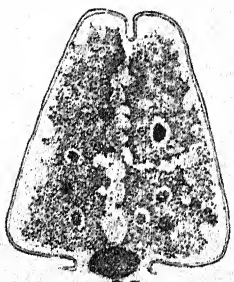
86.



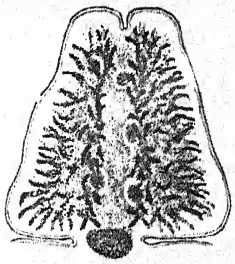
87.



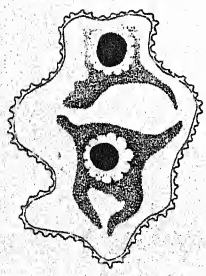
85.



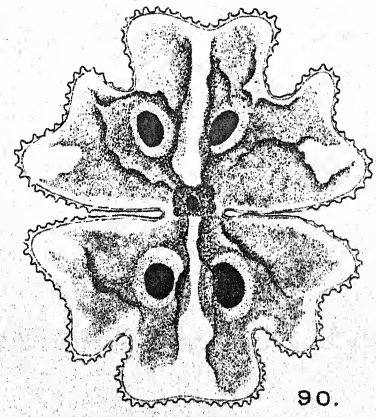
88.



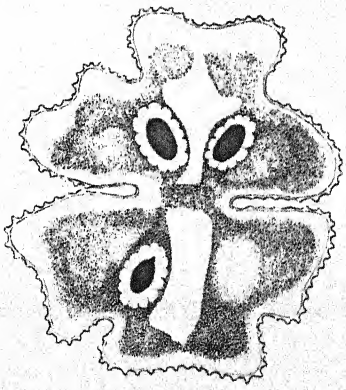
89.



92.

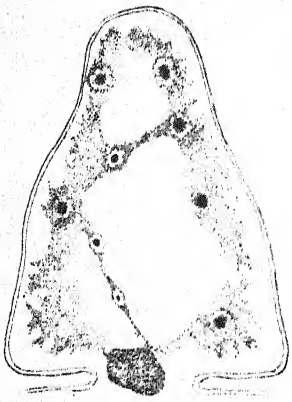


90.

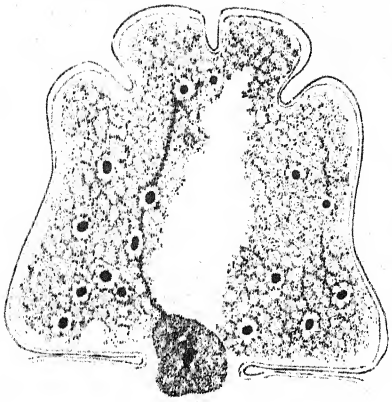


91.

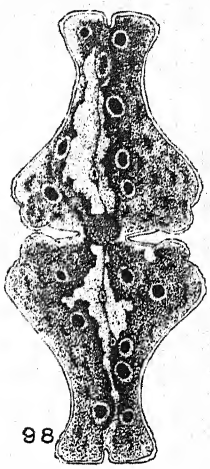
CARTER.N.—CHLOROPLASTS OF DESMIDS.



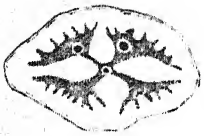
103.



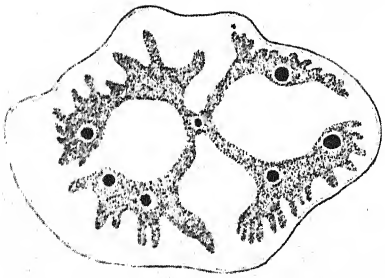
102.



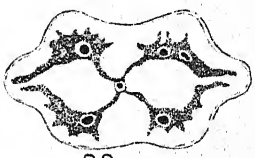
98.



97.

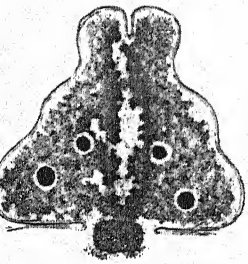


101.

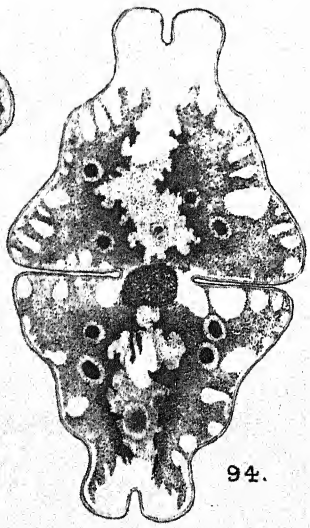


99.

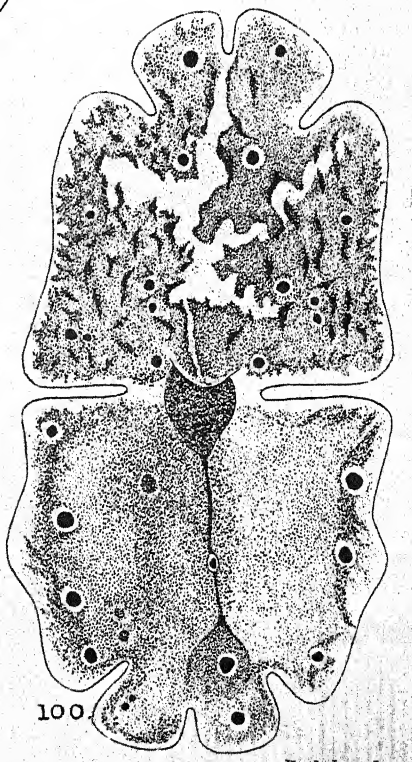
96.



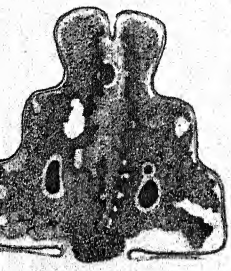
95.



94.

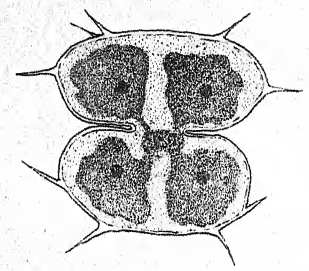


100.

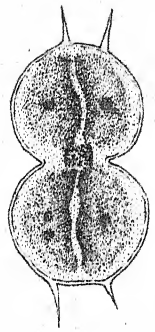


93.

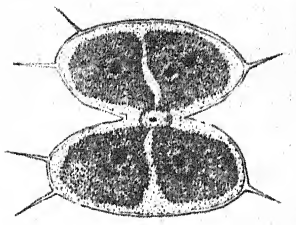
Huth, London.



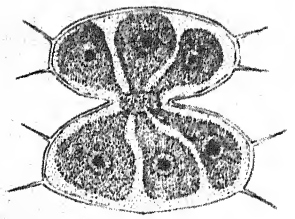
106.



107.

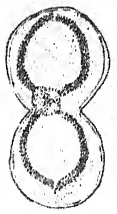


108.



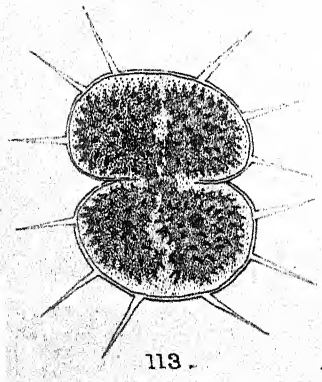
109.

110.

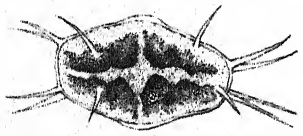


111.

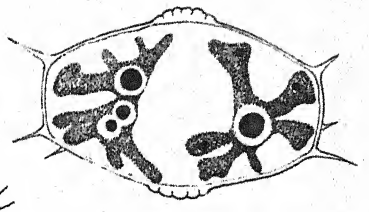
112.



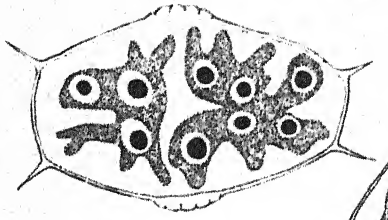
113.



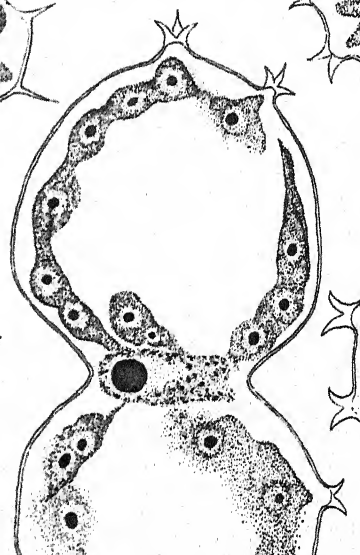
114.

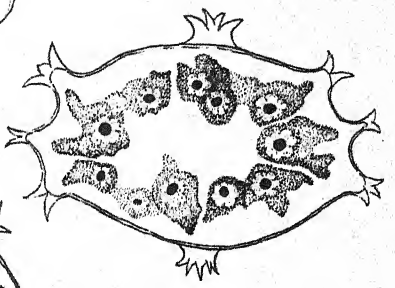


128.

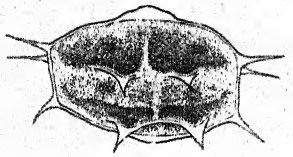


124.

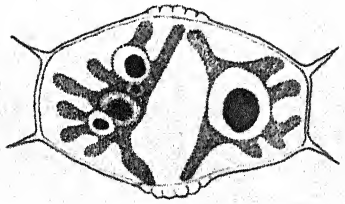




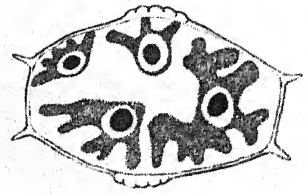
132.



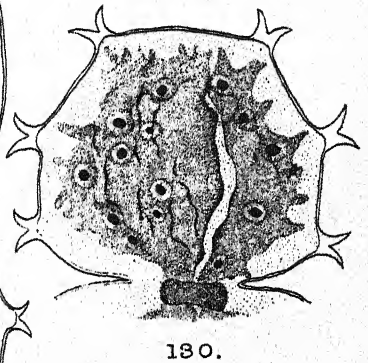
116.



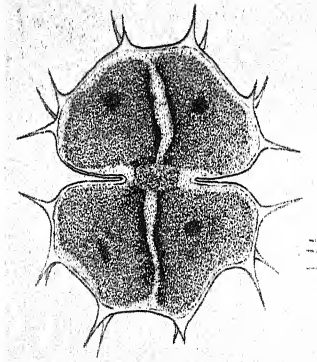
122.



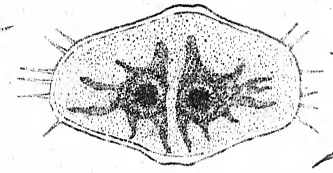
125.



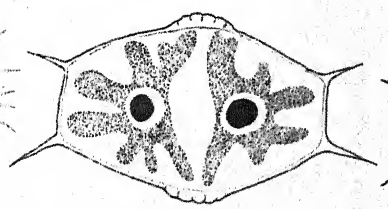
130.



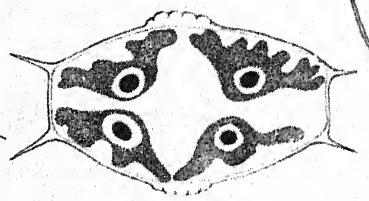
115.



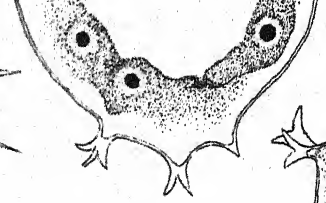
118.



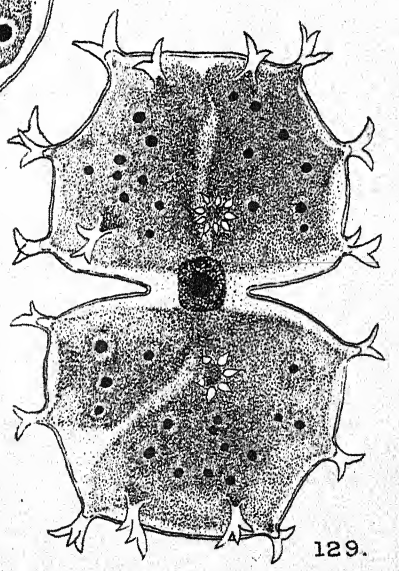
121.



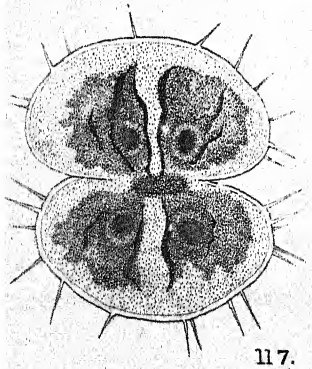
126.



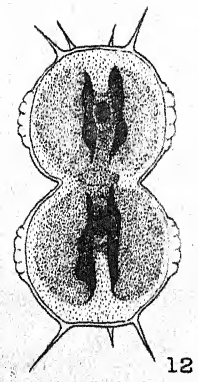
131.



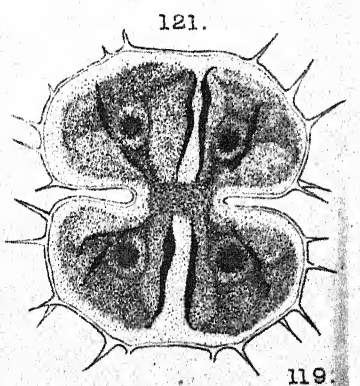
129.



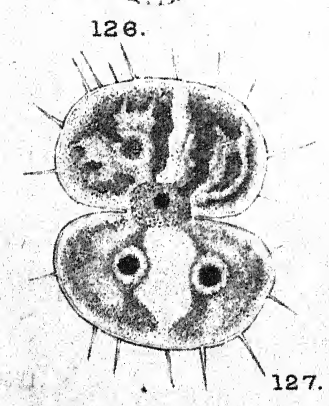
117.



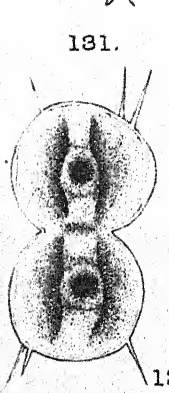
120.



119.



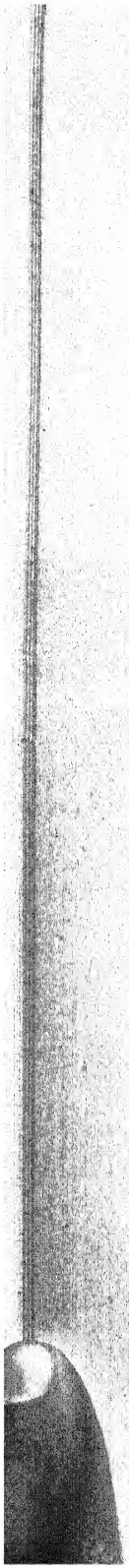
127.



128.

CARTER.N.—CHLOROPLASTS OF DESMIDS.

Huth, London.



Observations on the Anatomy of Ash-wood with Reference to Water-conductivity.

BY

M. G. HOLMES, B.Sc.

With seven Figures in the Text.

AN investigation into the anatomy of Ash-wood has been carried out on the lines of that described for Hazel-wood in a previous paper.[1] Attention was directed chiefly to the proportion of the water-conducting elements present in the wood. The results are presented graphically, and are intended to make possible a comparison between different parts of the same shoot as regards this character, as well as between shoots which differ in size and vigour.

Material.

As in the case of the Hazel, the Ash-wood here investigated was all wood of the first year. Three of the specimens, A3, A4, and A6, were typical stool shoots, long, thick, and unbranched, with long internodes and the scars of large leaves; they were cut on March 10, 1918, from separate Ash stools which had had one season's growth after coppicing. Specimen A8 was cut on May 18, 1918, from an Ash stool of four years' growth, being the upper part of one of the shoots, bearing several laterals of the previous season; three of these were chosen for comparison with the first-year stool shoots. They are much shorter than the latter, and lack some of the vigour characteristic of the juvenile form. At the season when A8 was cut, the buds had opened and the leaves were beginning to expand; the external appearance of the shoot is shown in Fig. 1.

In the Ash the opposite leaves of a pair are often not placed exactly level; sometimes in the stool shoots they are several centimetres apart. In these circumstances the position of the node was taken for convenience of comparison as the middle transverse plane between the upper limits of the two leaf scars, and the internodes were measured accordingly. The lengths of the whole shoots and of their internodes are compared in Fig. 2. The

[1] Holmes, M. G.: A Study in the Anatomy of Hazel-wood with Reference to Conductivity of Water. Ann. of Bot., vol. xxxii, 1918.

large axillary buds are placed well above the insertion of the leaf, and often below the large bud there is a much smaller supernumerary one. In the Ash shoot the terminal bud is generally present, but frequently it does not develop farther, and in the next season the strongest shoots grow out from the lateral buds near the apex. The apices of specimens A 3, A 4, and A 6 are compared in Fig. 3; it was found that the apical buds of A 3 and A 4, the shorter shoots, were strong, while that of A 6, the longer shoot, was weaker. In specimen A 8 is seen the result of the abortion of the terminal bud. That formed in 1916 failed to develop, and in 1917 six shoots grew out from lateral buds, getting less vigorous from the top downwards; a similar condition is repeated in the development of the buds of A 8 *a*, the strongest of these shoots, at the opening of the season of 1918. In the Ash a number of the shoots which do develop are not permanent; such weak shoots as A 8 *e* are likely to be suppressed very soon. In A 8 *b* the apex was dying back by the failure of the apical and also the lateral buds at the end.

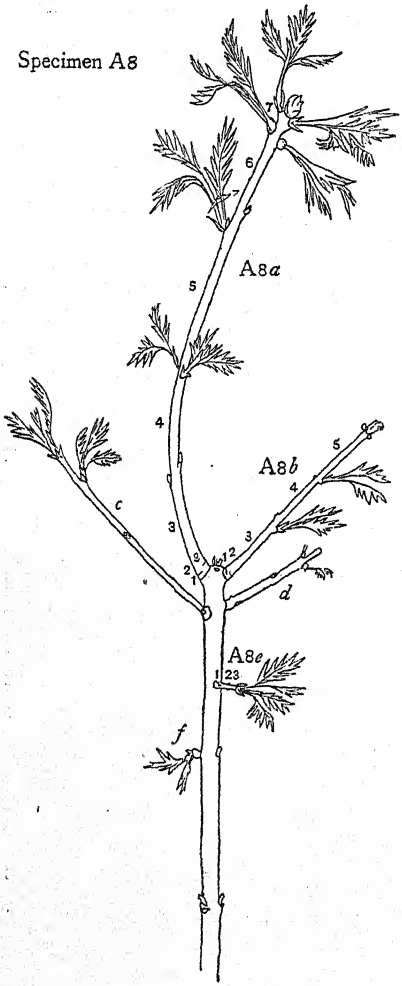


FIG. 1. The numbers refer to the internodes, reckoned from the base of each shoot respectively. The statistics of shoots A 8 *a*, A 8 *b*, A 8 *e*, are given in Fig. 7.

ANATOMY.

In the young and vigorous stool shoots there is a wide pith, especially in the middle part of the shoot. At the base, the wood cylinder is particularly wide between the cambium and the pith, and it decreases in width towards the apex relatively more than does either the pith or the cortex. There is a complete cylinder of wood in the terminal internode of each of these shoots, widest in A 3, internode 11, in the sense indicated above, and very narrow in A 6, internode 15. In A 8 the cambial activity had been resumed at the time that the specimen was cut, so that some new wood of the 1918 season was present; but this new spring wood was not taken into

account in the investigation, and the figures from which the graphs were drawn refer to the wood developed in the first season only, so that these graphs can be compared fairly with those given for A 3, A 4, and A 6, and also with those for the Hazel shoots,[1] H 7 to H 10. The wood cylinder for the first year is completely closed in all the sections cut from A 8, and is particularly wide between the cambium and the pith in internode 6 at the end of A 8 *a*.

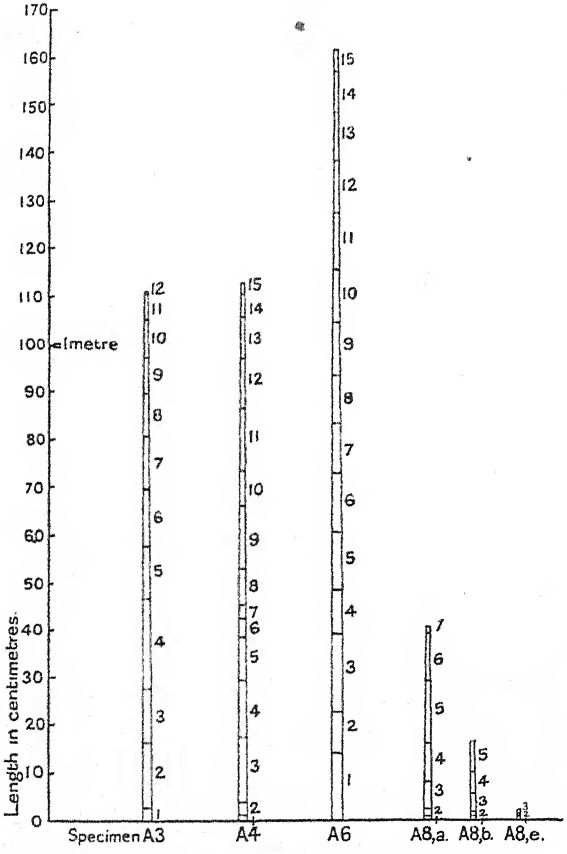


FIG. 2. The numbers refer to the internodes on each shoot respectively.

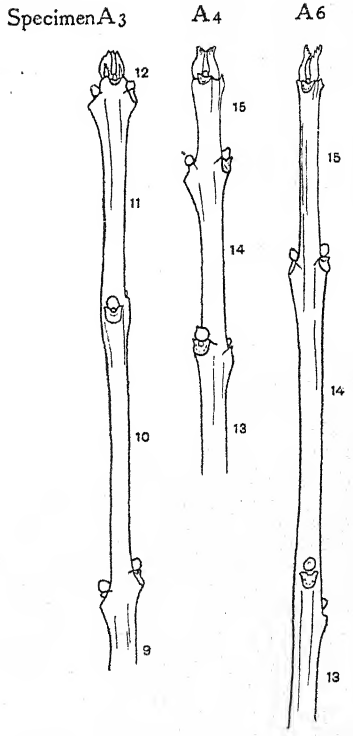


FIG. 3. The numbers refer to internodes, reckoned from the base of the shoot.

The water-conducting elements in Ash-wood are all vessels; there are no tracheides. The vessels are scattered among wood fibres, and are especially associated with wood parenchyma in vertical strands, the latter being most abundant near the autumnal limit of the cylinder.

The wood is crossed by numerous medullary rays, one or two cells wide, and often many cells deep. The vessels are mostly round or elliptical in transverse section, and have numerous bordered pits in their walls; they

[1] Holmes, M. G. : loc. cit.

are easily distinguished by their size and shape from the wood fibres, and by their walls, which are thicker, pitted, and more completely lignified than those of the latter. The small vessels near the cambium are very thick-walled, with a quite circular lumen. Spirally thickened vessels occur in the protoxylem next to the pith. In longitudinal section the vessels are seen to consist of short segments communicating by round holes, chiefly in radial oblique walls; the segments of the wide vessels may be four or five times

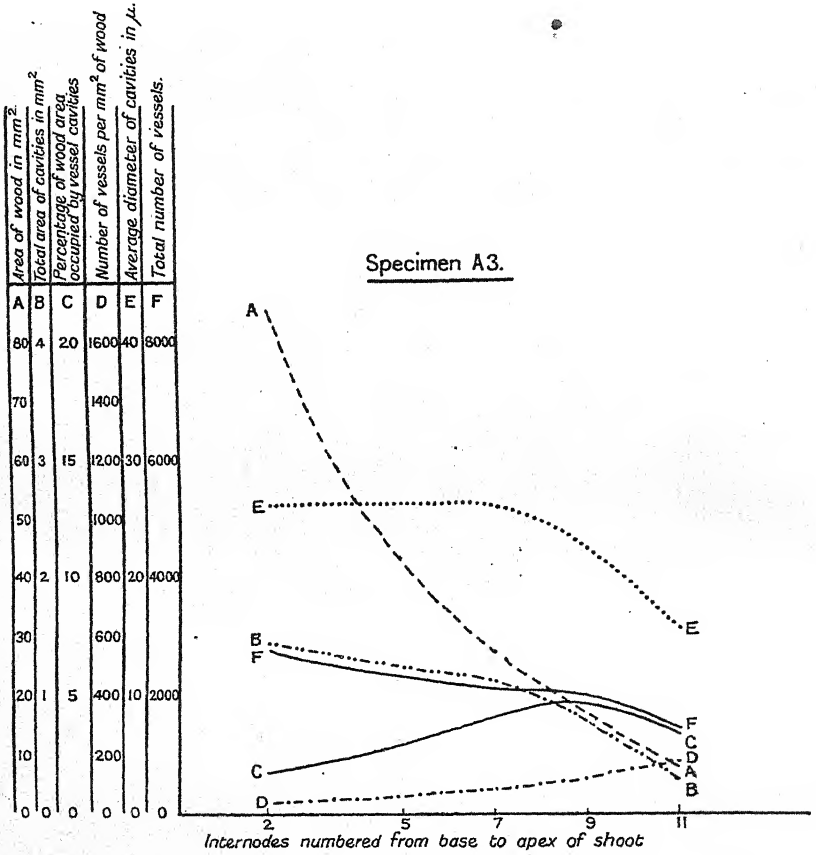


FIG. 4.

as long as they are wide, but are often shorter. The wood is richest in vessels immediately round the pith, especially in the four groups of primary xylem corresponding with the opposite and decussate phyllotaxy. In all except the smallest sections, where the widest vessels occur in these primary groups, there are wider vessels in the region of the wood just outside this part, and they get smaller again towards the periphery, and more scattered. Apart from the primary bundles, the distribution of the vessels is very uniform in most of the sections, especially at the lower levels.

METHOD.

The data as to the size, proportion, and distribution of the water-conducting elements were obtained in substantially the same manner as that described for the Hazel.[1] In many of the smaller sections it was found practicable to obtain the total number of the vessels by actual counting in the whole or a large part of the section.

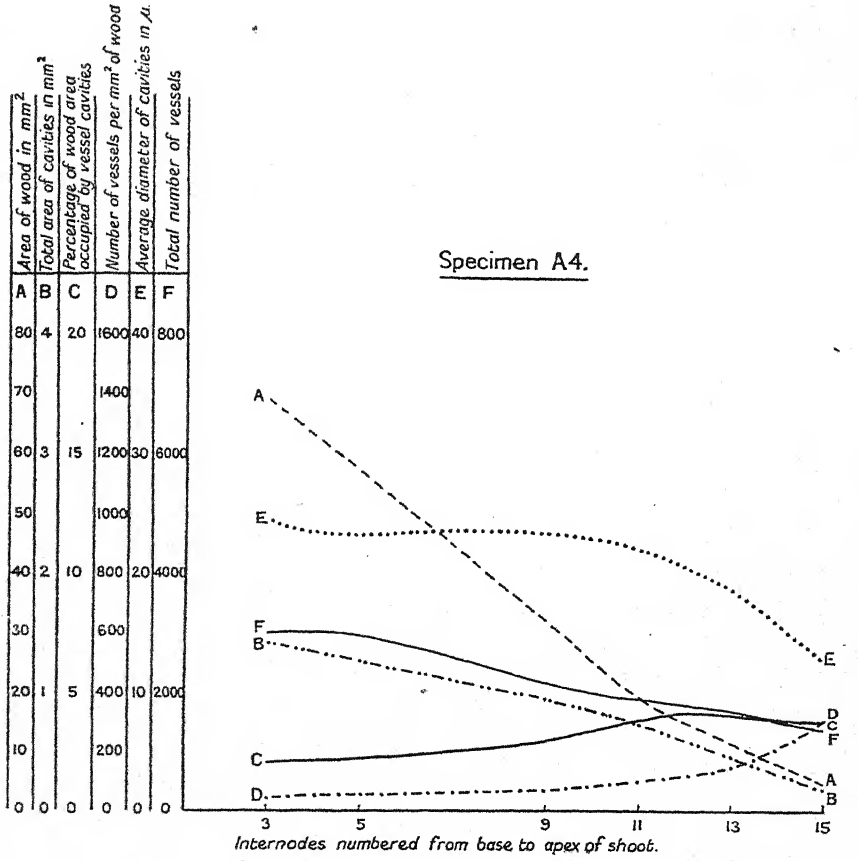


FIG. 5.

RESULTS.

The figures are presented in graphical form, a set of six curves being given for each shoot. These lines are lettered to correspond with those given for the Hazel shoots, and the vertical scales are the same. It has been found convenient, however, to double the horizontal scale, in which an equal interval was taken for each internode regardless of its length; for in the

[1] Holmes, M. G.: loc. cit.

stool shoots of the Ash the internodes are longer and fewer than in the Hazel stool shoots, and also each bears two leaves.

The total or absolute conductivity for water is represented, for purposes of comparison in this inquiry, by the area in square millimetres of all the vessels in each transverse section of the wood, taken at the middle points of the selected internodes. This is shown in curve B, and is obtained

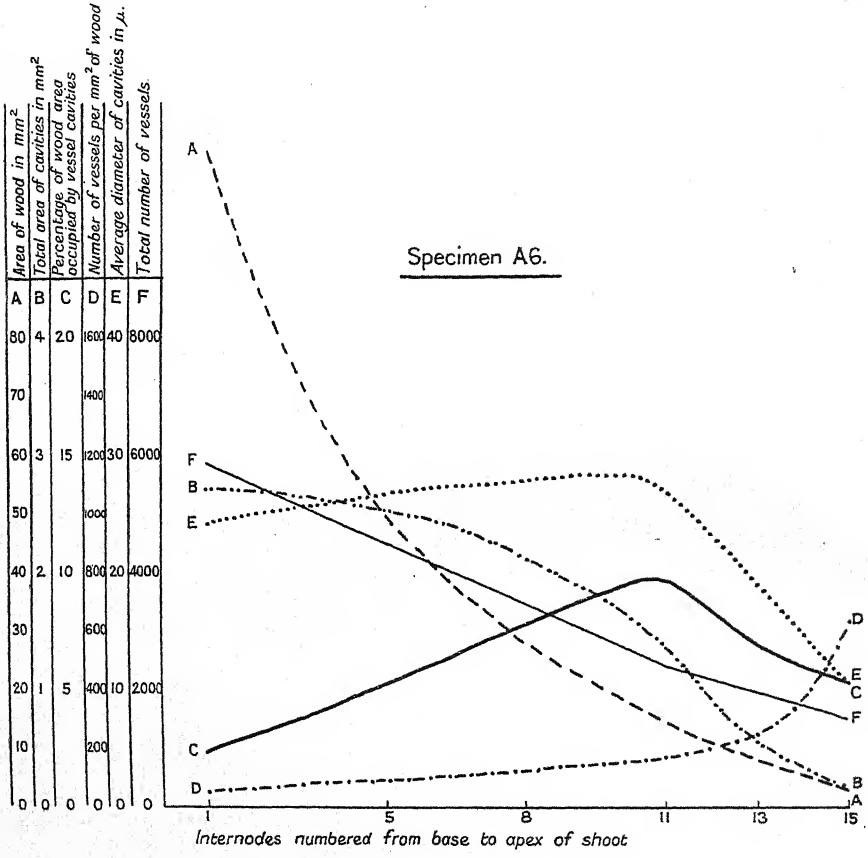


FIG. 6.

by calculation from curves F and E; it depends upon the total area of the wood, given in curve A, as well as upon its specific quality.

Curve A, giving the variation in area of the wood along each shoot, shows in general a very regular descent, rather steeper at the base; this is especially the case in the larger shoots, in which considerable support is necessary.

Curve F gives the total number of vessels present in the transverse section at the different levels in each shoot. It shows a fairly steady decline from base to apex; in A 8 *b* the decline is particularly steep.

Curve E gives the average diameter of the vessel cavities in the transverse section of the wood, measured in μ. The figure obtained for each section depends of course on the proportion of wide to narrow vessels, as well as on their actual widths. The curve keeps fairly level or may rise slightly from the base upwards for the greater part of the length of the shoot, and then falls again towards the apex. Only the fall is seen in the very small shoot A8*c*. Curve E reaches its highest point in

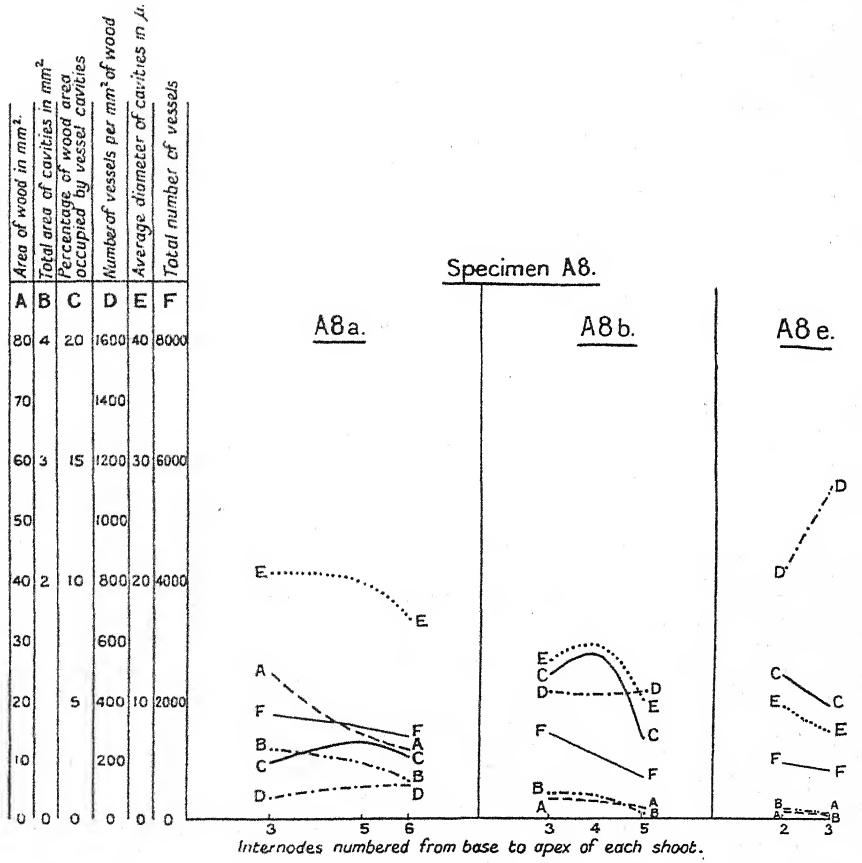


FIG. 7.

A6 at internode 10 (the highest calculated figure being 27·85 μ at A6, 8), but this of course is only an average value. In all sections narrow as well as wide vessels are present, but on the whole they are narrower in the smaller sections, and the rapid decline of the curve towards the apex is due to the absence of the larger vessels. The highest diameters recorded in each shoot for a single vessel were as follows:

Shoot No.	A6	A3	A4	A8*a*	A8*b*	A8*c*
Diameter of widest vessels in μ .	80	76	72	46	32	28

The vessels formed in the beginning of the second annual ring in A 8 are much wider than any present in the first ring, as is usual.

Curve B shows the total area of all the vessel cavities in each section, and represents the absolute water-conductivity. It is obtained by combining the data from curves F and E, and shows in general a corresponding decline from the base to the apex of the shoot. It is to be noticed that both the curves A and B reach a lower point in the terminal internode of A 6, in which the apical bud is weak, than in that of A 4, in which the bud is stronger. The higher figures for A 3, internode 11, are to be connected with the presence of two pairs of leaves above this point, instead of one pair as in A 4 and A 6; see Fig. 3. The same applies in the case of A 8 *a*, internode 6, as seen in Fig. 1. The values for curves A and B in the smaller shoots A 8 *b* and A 8 *e* are very much lower.

The specific conductivity for water is represented for comparative purposes by the percentage of the area of the wood occupied by the cavities of the vessels which it contains, in transverse section. This is shown in curve C. It depends upon the number of vessels present in a unit area of the wood as given in curve D, as well as upon their average width as discussed above for curve E. The figures for curve C are obtained most easily by combining the data from curves A and B.

Curve D gives the number of vessels per square millimetre of wood for each section. The numbers vary between 32 and 633 in the four larger shoots, with a higher range in the others. It will be seen that the curve rises from the base upwards for each shoot, indicating that the wood is richer in vessels near the apex, and richer in fibres near the base, where mechanical support is necessary. The ascent of the curve is especially rapid near the apex, because of the smaller size of the vessels in this part as well as their close packing. In this connexion the high values for curve D in A 8 *b* and A 8 *e* are due both to the much smaller proportion of mechanical elements in these weak shoots, and to the small diameter of the vessels.

Curve C serves as a measure of the specific water-conductivity of the wood, as explained above, in so far as it can be determined in transverse section. In general, the curve rises and then falls, the maximum occurring nearer the apex of the shoot than the base. In other words, up to a certain point the increase in the number of vessels, per unit area, makes the wood more efficient for water-conduction, its merely strengthening function being gradually reduced, but when the vessels get very small, the wood becomes less efficient again, their walls occupying a relatively larger area in proportion to their cavities. The rise and fall are shown in a general way by a comparison as a whole of the three curves for A 8; the figures are low for A 8 *a*, where there is a fairly high proportion of fibres, and fairly low again for A 8 *e*, where the vessels are very narrow. The sharp drop in curve C for the final internode in A 8 *b* is interesting in view of the failure on the part

of the buds at the apex of this shoot to develop. It was observed that this part of the shoot was alive at the resumption of cambial activity in the spring, for the section showed some development of new elements, though very slight as compared with that in the lower internodes. The highest value recorded for curve C was 9·5 per cent. at internode 11 in A 6, while the maximum for A 8 *b* at internode 4 reached 6·9 per cent. The lowest value was 1·68 per cent. at internode 2 in A 3. The percentage of 'conducting area', curve C, is lower at the base of A 3 than at the base of A 4, while the total 'conducting area', curve B, is about the same; this is because the larger area of wood in A 3, internode 2, contains a higher proportion of fibres.

Comparison with the results obtained for Hazel. There is a general resemblance between Ash and Hazel in the figures obtained for their stool shoots. The forms of the curves are similar, showing on the whole a decline in absolute conductivity with a rise in specific conductivity from the base of the shoot towards its apex, the latter falling again near the end.

The actual values for specific conductivity, curve C, however, are much lower for Ash than for Hazel, taken as a whole, the limits of variation for the shoots examined being as follows:

Plant.	*Ash.*	*Hazel.*
Range of values for curve C	1·68 to 9·5 per cent.	3·21 to 20·26 per cent.

This is in agreement with the results obtained by Professor Farmer,[1] for the two kinds of wood in question, by his experimental method of determining specific conductivity. His figures are quoted below:

Stool Shoots of	*Ash.*	*Hazel.*
Normal range of specific conductivity	14 ± 10	31 ± 9

Thus in both cases the figures obtained for Hazel-wood are about double those obtained for Ash-wood. In order to account anatomically for this difference we may compare the diameters of the water-conducting elements and their distribution, as shown in the present investigation. For the shoots examined the ranges of variation for the average and the actual diameters are as follows:

Stool Shoots of	*Ash.*	*Hazel.*
Range of values for curve E in μ	27·85 to 10·14	23·27 to 4·9
Range of actual diameters in μ	80 to 3	48 to 2

It is evident that, on the whole, the water-conducting elements are wider in Ash than in Hazel, so that the reason for the lower specific conductivity in Ash does not find its explanation in this feature. Turning to the distribution of these elements, the ranges of variation for the shoots examined are as follows:

Stool Shoots of	*Ash.*	*Hazel.*
Range of values for curve D	32 to 633	115 to 4,000

[1] Farmer, J. B.: On the Quantitative Differences in the Water-conductivity of the Wood in Trees and Shrubs, Parts I and II. Proc. Roy. Soc., B., vol. xc, 1918.

Thus on the whole Hazel-wood has a greater number of water-conducting elements per square millimetre than Ash-wood, which more than compensates for the smaller diameter of these elements and their greater resistance to the flow of water on this account. The relatively low specific conductivity of Ash-wood must be attributed to its relative poverty in vessels. It is to be understood that these observations apply to wood of the first year only, and that a still greater range of variation might, and probably would, be found for some of the characters—for instance, in those forming the basis of curve E—by examining a larger number of examples; but I am inclined to think that the general range for specific conductivity would not be altered materially, and that the relation between Ash- and Hazel-wood in this respect would remain as indicated above.

Summary.

The results obtained from a quantitative investigation of the constitution of Ash-wood in young shoots, with special reference to vessel content, have been discussed, and compared with similar data previously obtained for Hazel-wood. It is shown that on the whole there is a fall in absolute water-conductivity and a rise in specific conductivity from the base of a shoot to its apex, in both kinds of wood examined, and that, in general, the figures for specific conductivity are lower in Ash than in Hazel. In the latter the water-conducting elements are more numerous than in the former, though on the whole they are not so wide.

My thanks are due to Professor Farmer, of the Imperial College, South Kensington, for his suggestion of this work and his help in carrying it out, and to Professor Potter, of Armstrong College, Newcastle-on-Tyne, where part of the work was undertaken.

NOTE.

NOTE ON THE DURATION OF THE PROTHALLIA OF LASTRAEA FILIX-MAS, PRESL.—I venture to record here some observations on prothallia of *Lastraea Filix-mas*, which seem to indicate a greater vitality and more prolonged duration than have been generally attributed to prothallia.

In August, 1912, I desired to obtain some early stages of fern prothallia for class purposes. To this end, I used a tank with a glass front, and covered the bottom with pieces of coke partly immersed in water. On these were laid some mature fronds of *Lastraea*, and the top covered over with slate slabs. The conditions for germination seem to have been successfully met, and by early October the pieces of coke were covered by myriads of prothallia in all the early stages of development.

These were in due course used for demonstration purposes, and the tank dismantled. I retained, however, two or three pieces of the coke, and placed them in a glass basin with water, and covered the top with a glass plate. The basin was then placed on a shelf in the laboratory and for some time forgotten.

It was not until March, 1914, after an interval of about twenty months from the sowing of the spores, that I remembered about these prothallia, and re-examined them. Fortunately the water had not dried up, thanks to the circumstance that the edge of the basin was ground, and the covering plate fitted fairly closely.

The appearance then presented by the contents of the basin was so remarkable, that at the first glance I supposed that the crop of prothallia had been succeeded by a crop of moss. For in the feeble illumination of the shelf, each prothallium had grown vertically upwards, covering the flanks of the coke lumps as thickly as they could stand, and resembling miniature forests on the sloping surfaces. The average height was about 15 millimetres, and the breadth from 1 millimetre to 1½. From the surface turned away from the light (invariably the morphologically under surface) innumerable rhizoids attached the vertical prothallia like guy-ropes to the surface of the coke, and the edges bristled with the characteristic mucilage cells. While the prothallia were light green through the greater part of their length, the bases were brown through the death of the cells. I could find no fungi, however, on the decadent portion.

On microscopic examination, I could find no trace of archegonia on any of these depauperated prothallia, but the whole of the under surface was studded over with antheridia, to the number of many hundreds on each. Near the apex were immature antheridia, then farther back antheridia which released the antherozoids on access of water, and in the older parts antheridia which had dehisced. I calculated that upon the one prothallium on which I attempted a count, there must have been from 700 to 1,000 antheridia from base to apex.

I now transplanted some of these prothallia, laying them flat with the under surface downwards, on suitable soil in an earthenware saucer, and placed them under a bell-

jar on a window ledge. They gradually established themselves, growing forward quite normally, broadening, and deepening the colour as they grew. By July, young plants began to appear, which showed their second leaf in August. I then made a second transplantation with a similar result.

The original stock in the glass basin was replaced in its old situation on the shelf in order to discover how long the prothallia could retain their vitality under these conditions.

I am now able to state that some of these prothallia have remained alive through the years 1915–16–17 into the current year, that is, for nearly six years from the sowing of the spores. They have gradually languished, however, and many have died off altogether, but even last spring I found young antheridia immediately behind the apices of the surviving prothallia. I was not able to make a successful transplantation from the stock this year, and at the time of writing all the green apices have disappeared.

The points which seem to me from these observations worth record are (1) the narrowing of the prothallus and the assumption of the vertical habit in the feeble illumination, (2) the rigid maintenance of the morphological dorsiventrality in this condition, (3) the recovery of the capacity to produce archegonia on transplantation to more suitable conditions, (4) the great tenacity of life under unfavourable conditions, and (5) the comparative immunity from fungal attack.

R. W. PHILLIPS.

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR.
September, 1918.

The Floras of the Outlying Islands of New Zealand and their Distribution.

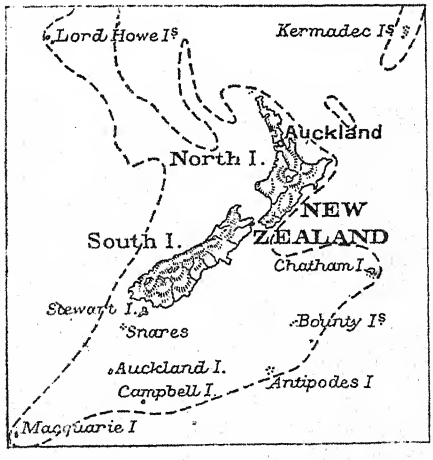
BY

J. C. WILLIS, M.A., Sc.D., F.R.S.,

European Correspondent of the Botanic Garden, Rio de Janeiro.

With two Maps and twenty-one Tables in the Text.

IN this paper I shall deal chiefly with the smaller islands that lie at some distance from New Zealand, but on the same submarine plateau, especially with the Kermadecs, Chathams, and Aucklands, following up the work on the taxonomic distribution of the New Zealand flora given in a series of preceding papers (6 to 11). In one of these (8) I have already shown that one may prophesy, with the aid of age and area, that the plants that reach these islands will on the whole be the oldest, and therefore the most widespread, in New Zealand, and find, on examination of the facts, that the prophecy is completely borne out.

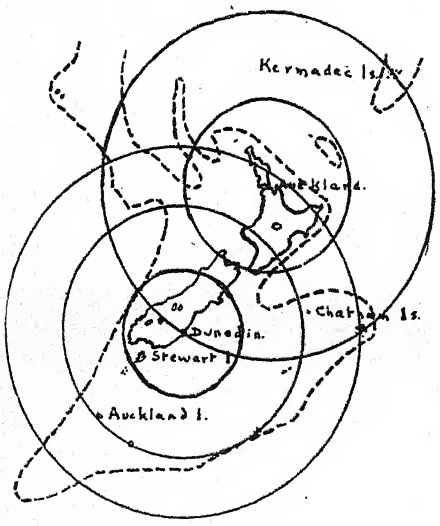


New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

My authority for the floras continues to be in the main Cheeseman's New Zealand Flora (2), supplemented by his later lists in Chilton's 'Subantarctic Islands' (3), and by Cockayne's lists (4). As I have already pointed out, the detailed completion of a flora makes but slight differences in the final result. This was very strikingly shown in the additions to the Ceylon flora made in a short paper subsequently published (13), and in those to the flora of Stewart Island given in the appendix to my last paper (11). In neither instance was any serious difference made by the addition in the one case of 110, in the other of 71, further species. By

keeping to the larger floras and papers it must not be forgotten that I gain the very considerable advantage of dealing with a flora worked up by one or by a few persons, and consequently one which is fairly uniformly treated in its conception of specific forms, &c.

The second map-diagram of my last paper, reproduced here, represents an imaginary distribution of the plants of the northern and southern invasions of New Zealand already dealt with, and supposed, for the sake of simplicity, to centre in Auckland (city) and Dunedin. A very brief consideration of it shows that while on the whole the plants of Stewart Island will be older in New Zealand itself than the general average of the plants of New Zealand, those of the outlying islands will be on the whole at least as old, and in most cases probably older, as they would have to start very early from New Zealand to reach the islands before they were cut off. The Kermadecs and Aucklands, however, having been to some extent in the track of invasions, may of course contain species which arrived there too late to reach New Zealand at all, or only just reached it at the last minute before it was cut off. The Chathams, on the other hand, must clearly have received all, or nearly all, their flora by way of New Zealand; no invasion can have passed that way. We shall start from this conception of the great relative age of the floras of the outlying islands as a fundamental fact, and study the floras of these islands by the method of prediction and verification, just as was done in the case of Stewart.



New Zealand and islands to show imaginary distribution from Auckland and Dunedin.

One will expect to find that there is what one may perhaps term a selection list (the Scottish term 'short leet' exactly expresses it) of the (in New Zealand) older families, from which Stewart as the nearest island will select practically all, while the more outlying islands will have fewer of them. But the families of these islands should be all included in the Stewart list, unless they be the later arrivals of the northern or southern invasions, or families which have their maximum development in the centre of New Zealand, from which it is less distance to the Chathams than to Stewart.

Now if Natural Selection were the determinant, one would hardly expect, when one considers the extremely different climatic, geological, and other conditions of these widely separated islands, that they would, so to speak, choose their floras from just the same families. One would anticipate finding considerable differences between them, and that more of the other important families of New Zealand would occur, or that the islands would contain relicts belonging to some of the less important families in New Zealand.

(1) We shall therefore expect to find that the islands have *proportionately* more in common with Stewart in the matter of families than with New Zealand proper.

TABLE I.

Occurring in	*Aucklands.*	*Chathams.*	*Kermadecs.*	
	32 / 35 %	48 / 52 %	37 / 40 %	of 91 New Zeal. fams.
	32 / 53 %	41 / 68 %	28 / 46 %	of 60 Stewart fams.

Thus all the islands, even the Kermadecs, contain a considerably higher percentage of Stewart families than they do of New Zealand families. All the Auckland families occur in Stewart, 41 out of 48, or 85 per cent., of the Chatham families, and even 28 out of 37, or 75 per cent., of the Kermadec families.

(2) We shall expect to find that a large part of the families in the different islands are the same, and that most of them are selected from the Stewart list. The following table gives, in ordinary type, the 60 Stewart families, with the islands in which they occur. Those not occurring in Stewart are printed in capitals.

TABLE II.

Fam.	*Spp. in Stewart Island.*	*Fam.*	*Spp. in Stewart Island.*
Ranunculaceae	10 A C	Droseraceae	4 A
Magnoliaceae	1 —	Haloragidaceae	13 A C K
Cruciferae	3 A C K	Myrtaceae	4 A C K
Violaceae	6 C K	Onagraceae	13 A C
Pittosporaceae	1 K	CUCURBITACEAE	— K
Caryophyllaceae	4 A C	Ficoideae	3 C K
Portulacaceae	2 A	Umbelliferae	14 A C K
Elatinaceae	1 —	Araliaceae	7 A C K
Hypericaceae	1 —	Cornaceae	1 C
Malvaceae	2 C	Rubiaceae	17 A C K
Linaceae	1 C	Compositae	54 A C K
Tiliaceae	4 —	Stylidiaceae	4 A
Geraniaceae	4 A C K	Goodeniaceae	1 K
RUTACEAE	— K	Campanulaceae	3 A C K
RHAMNACEAE	— C	Ericaceae	2 —
ANACARDIACEAE	— C K	Epacridaceae	10 A C
Coriariaceae	2 C K	Primulaceae	1 A C K
LEGUMINOSAE	— C K	Myrsinaceae	4 A C K
Rosaceae	7 A C K	Apocynaceae	1 —
Saxifragaceae	3 —	Loganiaceae	1 —
Crassulaceae	2 A C	Gentianaceae	4 A C

TABLE II (*continued*).

Fam.	*Spp. in Stewart Island.*				*Fam.*	*Spp. in Stewart Island.*			
Boraginaceae	3	A	C		Loranthaceae	1		—	
Convolvulaceae	3		C	K	Euphorbiaceae	1		C	K
SOLANACEAE	—		C	K	Urticaceae	1	A	C	K
Scrophulariaceae	13	A	C	K	Orchidaceae	21	A	C	K
MYOPORACEAE	—		C	K	Iridaceae	2		C	
Lentibulariaceae	1		—		Liliaceae	12	A	C	K
Labiatae	1		C		Juncaceae	8	A	C	
Plantaginaceae	3	A			PALMAE	—		C	K
Illecebraceae	1		—		TYPHACEAE	—			K
Chenopodiaceae	2		C	K	Naiadaceae	4		C	
Polygonaceae	3	A	C	K	Centrolepidaceae	2	A		
PIPERACEAE	—		C	K	Restionaceae	2		C	
Chloranthaceae	1			K	Cyperaceae	44	A	C	K
Thymelaeaceae	3		C		Gramineae	35	A	C	K

Thus of the 60 Stewart families, 49 (or 81 per cent.) occur in the other islands, while of the 31 families that occur in New Zealand and do not occur in Stewart, only 10 (or 32 per cent.) occur in the islands. Of these 10, Rutaceae, Rhamnaceae, Anacardiaceae, Cucurbitaceae, Myoporaceae, Piperaceae, Palmae, and Typhaceae, are enumerated in the list of the northern invasion given on p. 356 of 10, and Solanaceae is given as probably belonging to that invasion, so that only Leguminosae remains. This is represented in the Kermadecs by *Canavalia*, a genus that does not occur in New Zealand, and in the Chathams by *Sophora tetraptera*, a species which should in all probability be found on Stewart.

It is thus clear that the prophecy made above is borne out by the facts. A mere glance at the table suffices to show how many of the families occur in two or more groups of islands, and we have seen that most of them also occur in Stewart.

(3) As we have already seen, in dealing with Stewart Island, the largest families (in a given country) will on the whole be the oldest (in that country). We may therefore predict that the families that reach all three groups of islands will on the whole be the largest, both in New Zealand as a whole, and in Stewart. Those reaching two groups will be next in size, and then will follow those reaching only one, and those reaching none. An examination of the facts gives us

TABLE III.

Reaching	*The families contain (in New Zealand as a whole),*
3 islands.	221, 119, 113, 113, 61, 57, 47, 22, 22, 20, 19, 17, 17, ‖ 11, 11, 9, 8, 8, 1.
2 „	50, 31, 31, 26, 25, 25, 20, 17, ‖ 15, 12, 10, 10, 5, 4, 3, 3, 2, 2, 1, 1.
1 island.	19, ‖ 15, 13, 7, 7, 7, 6, 6, 5, 5, 3, 3, 3, 3, 2, 2, 2, 2, 1, 1.
No islands.	‖ 10, 8, 6, 6, 6, 6, 4, 4, 4, 4, 3, 3, 2, 2, 2, 2, 2, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1.

The mark ‖ is placed at the position of the average size of family in New Zealand (15·2 species).

A similar comparison for Stewart Island shows that the size of the families in that island, according to the number of groups they reach, is:

TABLE IV.

Reaching	*Size of Families in Stewart Island.*[1]
3 islands.	54, 38, 30, 21, 17, 14, 13, 13, 13, 10, 7, 7, ‖ 4, 4, 3, 3, 3, 1, 1.
2 ,,	12, 10, 10, 8, ‖ 6, 4, 4, 3, 3, 3, 2, 2, 2, 1.
1 island.	‖ 4, 4, 4, 3, 3, 2, 2, 2, 2, 1, 1, 1, 1, 1, 1.
No islands.	‖ 4, 3, 2, 1, 1, 1, 1, 1, 1, 1, 1.

A mere glance at these figures is enough to show that the prophecy is borne out by the facts; but we may put it in tabular form thus:

TABLE V.

	New Zealand Families.			*Stewart Families.*[1]		
Reaching	*Fams.*	*Spp.*	*Average size.*	*Fams.*	*Spp.*	*Average size.*
3 islands	19	896	47·1	19	256	13·4
2 ,,	20	293	14·6	14	70	5·0
1 island	20	112	5·6	16	34	2·1
No island	32	91	2·8	11	17	1·5

(4) Just as we predicted in the case of Stewart that the New Zealand families omitted would be on the whole the smallest, so here we may predict that the missing Stewart families will on the whole be the smallest, a prophecy which is clearly borne out by the facts in Tables IV and V.

(5) One will expect the Aucklands to contain many of the families of the southern invasion—they contain all but Naiadaceae. One will expect the Chathams also to contain many, and on the whole to lack the smaller ones. The smallest, those with less than 11 species, are Portulacaceae, Droseraceae, Stylidiaceae, Plantaginaceae, and Centrolepidaceae, and it is exactly these which are missing in the Chathams. The bulk of the invasion, if not all of it, is missing in the Kermadecs.

(6) One will expect the Chathams to contain many of the families of the northern invasion, and perhaps the Kermadecs also, but, as we have already seen (10, p. 358), they do not seem to have lain in the track of the main invasion. Examination of the facts shows that the Chathams contain only 9 of the 33 families, and the Kermadecs 11, while Stewart itself only contains 8 and the Aucklands 3. It is thus evident that old though it was, the northern invasion did not in general get very far south. This may have been due to the species reaching their climatic boundary, in some cases at any rate, though my experience of the surprising degree of cold which many 'tropical' species bear in southern Brazil, and the fact that so many tropical species go very far south in New Zealand, makes me hesitate to say that a species has reached its climatic limit. Given slow enough progress, a species may travel into climates to which it would seem quite unsuitable.

[1] Note the much greater proportion of the Stewart families reaching islands, even three islands. The Stewart families are on the whole older.

(7) One may also predict that the outlying islands will have proportionately many more families in common with one another than they have with New Zealand.

TABLE VI.

	Kermadecs.	*Chathams.*	*Aucklands.*	*New Zealand.*
Kermadecs in common with	—	31 64%	19 59%	37 40%
Chathams ,, ,, ,,	31 83%	—	27 84%	48 52%
Aucklands ,, ,, ,,	19 51%	27 56%	—	32 35%

Even the Aucklands have a greater proportion in common with the Kermadecs than any of the islands but the Chathams have with New Zealand.

(8) We may now go on to deal in the same way with the genera, and may begin by predicting that they will be chiefly selected from the list of genera found in Stewart Island. The result of testing this prediction is given in the following table, which gives the Stewart list, with the number of species in Stewart, and the islands in which the genera are found. The genera that occur in the islands and New Zealand and have not been found in Stewart are printed in capitals.

TABLE VII.

	Clematis	1	—		
	Ranunculus	8	A	C	
	Caltha	1	—		
	Drimys	1	—		
	Cardamine	1	A	C	K
	Lepidium	2	A	C	
	Viola	2		C	
	Melicytus	2			K
	Hymenanthera	2	—		
10	Pittosporum	1			K
	Stellaria	1	A	C	
	Colobanthus	2	A	C	
	Spergularia	1	—		
	Claytonia	1	—		
	Montia	1	A		
	Elatine	1	—		
	Hypericum	1	—		
	Plagianthus	2		C	
	Aristotelia	3	—		
20	Elaeocarpus	1	—		
	Linum	1		C	
	Geranium	3	A	C	K
	Pelargonium	1		C	
	OXALIS	—			K
	MELICOPE	—			K
	POMADERRIS	—		C	
	CORYNOCARPUS	—		C	K
	Coriaria	2		C	K
	SOPHORA	—		C	
30	Rubus	3	—		
	Geum	1	A		
	Potentilla	1		C	
	Acaena	2	A	C	K
	Donatia	1	—		
	Carpodetus	1	—		
	Weinmannia	1	—		
	Tillaea	2	A	C	
	Drosera	4	A		
	Haloragis	4	A		K
40	Myriophyllum	4	—		
	Gunnera	4		C	
	Callitriche	1	A	C	K
	Leptospermum	1		C	
	Metrosideros	2	A		K
	Myrtus	1	—		
	Epilobium	11	A	C	
	Fuchsia	1	A		
	SICYOS	—			K
	Mesembryanthemum	1		C	K
50	Tetragonia	2		C	K
	Hydrocotyle	5		C	K
	Azorella	1	A		
	Actinotus	1	—		
	Apium	1	A		K
	Oreomyrrhis	1		C	
	Crantzia	1		C	
	Aciphylla	1		C	
	Ligusticum	3	A		
	DAUCUS	—		C	
60	Aralia	1	—		
	Panax	4	A		K
	Schefflera	1	—		
	Pseudopanax	1		C	
	COROKIA	—		C	
	Griselinia	1	—		
	Coprosma	13	A	C	K
	Nertera	3	A		
	Asperula	1	—		
	Lagenophora	2	A	C	K
70	Brachycome	2	—		
	Olearia	8	A	C	
	Celmisia	7	A		

TABLE VII (*continued*).

	Gnaphalium	4	A	C	K		Parietaria	—			K
	Raoulia	3		—			Dendrobium	1		—	
	Helichrysum	4	A	C			Earina	2		C	
	Cassinia	2	A				Sarcochilus	1		C	
	Craspedia	1		—			Thelymitra	2	A	C	
	Siegesbeckia	—			K	140	Microtis	1		C	K
	Bidens	—			K		Prasophyllum	1	A		
80	Cotula	4	A	C	K		Pterostylis	3		C	
	Abrotanella	2	A				Acianthus	—		C	K
	Erechtites	6	A	C			Lyperanthus	1	A		
	Senecio	6		C	K		Caladenia	2	A	C	
	Microseris	1		—			Chiloglottis	1	A	C	
	Taraxacum	1		—			Corysanthes	5	A	C	
	Sonchus	2	A	C	K		Gastrodia	1		C	
	Phyllachne	2	A				Libertia	2		C	
	Oreostylidium	1		—		150	Rhipogonum	1		C	
	Forstera	1		—			Enargea	1		—	
90	Selliera	1		—			Cordyline	1			K
	Pratia	1	A	C			Astelia	3	A	C	
	Lobelia	—		C	K		Phormium	2		C	
	Wahlenbergia	2		C	K		Bulbinella	2	A		
	Gaultheria	2		—			Arthropodium	1		—	
	Pentachondra	1		—			Herpolirion	1		—	
	Cyathodes	2	A	C			Rostkovia	—	A		
	Leucopogon	1		C			Juncus	7	A	C	
	Archeria	1		—		160	Luzula	1	A	C	
	Dracophyllum	5	A	C			Rhopalostylis	—		C	K
100	Samolus	1	A	C	K		Typha	—			K
	Myrsine	4	A	C	K		Triglochin	1		C	
	Parsonsia	1		—			Potamogeton	2		—	
	Mitrasacme	1		—			Zostera	1		—	
	Gentiana	3	A	C			Centrolepis	1		—	
	Liparophyllum	1		—			Gaimardia	1	A		
	Myosotis	3	A	C			Lepyrodia	—		C	
	Ipomoea	—			K		Leptocarpus	1		—	
	Calystegia	2		C	K	170	Hypolaena	1		—	
	Dichondra	1		C			Eleocharis	3		C	
110	Solanum	—		C	K		Scirpus	7	A	C	K
	Glossostigma	1		—			Carpha	1	A		
	Veronica	5	A	C	K		Schoenus	3		C	
	Ourisia	5		—			Cladium	3		C	
	Euphrasia	2		—			Gahnia	1		—	
	Utricularia	1		—			Oreobolus	2	A		
	Myoporum	—		C	K		Uncinia	9	A	C	
	Mentha	1		C			Carex	15	A	C	K
	Plantago	3	A			180	Ehrharta	1			
	Scleranthus	1		—			Microlaena	2	A		
120	Rhagodia	—			K		Agrostis	1	A		
	Chenopodium	1		—			Hierochloe	2	A	C	
	Atriplex	1		C			Oplismenus	—			K
	Salicornia	—		C			Deyeuxia	5	A	C	K
	Polygonum	—		C			Dichelachne	1		C	
	Rumex	1	A	C	K		Deschampsia	2	A	C	
	Muehlenbeckia	2		C			Trisetum	1	A	C	
	Piper	—		C	K		Danthonia	6	A	C	
	Peperomia	—			K	190	Arundo	1		C	
	Ascarina	1			K		Agropyrum	1			K
130	Pimelea	1		C			Poa	8	A	C	K
	Drapetes	2		—			Atropis	1	A		
	Loranthus	1		—			Asprella	1		—	
	Euphorbia	1		C			Festuca	2		C	
	Urtica	1	A	C							

Thus we find, on summing up, that while New Zealand as a whole has 329 genera, of which 169 occur in Stewart and 160 do not, 114 of the

former, or 67 per cent., occur in the islands, while of the latter only 26 (the genera in capitals) occur, or 17 per cent., an enormous difference. The prophecy made above is thus fully borne out by the facts.

Further, of the 26, 11 occur only in New Zealand and the Kermadecs, 7 in the Chathams, 7 in both Kermadecs and Chathams, and one only (*Rostkovia*) in the Aucklands and the South Island of New Zealand. This was probably a late arrival of the southern invasion. 15 of the 26 genera belong to families enumerated in the northern invasion (10, p. 356), and all but *Rostkovia* are distinctly northern types, most of which probably took part in the northern invasions.

(9) Just as with the families, we may predict that those genera will on the whole be the largest in number of species, whether in New Zealand as a whole, or in Stewart Island, which reach three of the outlying groups of islands. Then will follow those reaching two islands, one island, and no islands at all. Testing this we find:

TABLE VIII.

Reaching	*Gen.*	*Spp. in Stewart.*	*Average.*	*Spp. in N.Z.*	*Average.*
Stewart and 3 groups	17	78	4·5	293	17·2
,, ,, 2 ,,	39	132	3·3	396	10·1
,, ,, 1 group	58	99	1·7	294	5·0
,, ,, no ,,	55	74	1·3	162	2·9
Neither Stewart nor other islands	133			204	1·5
Other islands, but not Stewart	27			43	1·5

(10) Just as with the families, one may predict that the islands will have proportionately more genera in common with one another than they have with New Zealand.

TABLE IX.

	Kermadecs.	*Chathams.*	*Aucklands.*	*New Zealand.*
Kermadecs in common with	—	32	21	62
		32 %	30 %	19 %
Chathams ,, ,, ,,	32	—	44	98
	51 %		64 %	29 %
Aucklands ,, ,, ,,	21	44	—	68
	33 %	44 %		20 %

Again the facts bear out the prophecy.

(11) One may predict that the genera which occur in Stewart and do not occur in the islands will on the whole be the smaller genera of Stewart. The average number of species in a genus in Stewart is 2·2, and of the 55 genera that do not occur in the islands 44 have one species in Stewart Island, six have 2, and the others 3, 3, 3, 4, and 5, or an average of 1·3 species per genus.

(12) We may now go on to the actual species, and predict that these will also be largely selected from the Stewart list. It would occupy too

much space to give the whole list over again (cf. 11, p. 27), but of the 382 species so far recorded from Stewart, no fewer than 153, or 40 per cent., are found in the islands, whereas of the 1,301 species found in New Zealand as a whole, only 199 occur on the islands, or 15 per cent. Thus there are only 46 species on the islands, other than local endemics or species which do not occur at all in New Zealand, which do not occur in Stewart, against 153 which do.

(13) We may predict that the families which occur in Stewart but have no species on the islands will on the whole be small (in Stewart). The average size of a family in Stewart is 6·3 species, and those families that contain no species on the islands contain in Stewart 4, 3, 2, 1, 1, 1, 1, 1, 1, 1, 1 species, or all below the average size in Stewart.

(14) We may also predict in this connexion that the islands will have very many species in common between themselves, and this also proves to be the case. The Kermadecs have a total flora of 71, of which 19 occur nowhere else, leaving 52, of which 30 occur in the Chathams and 5 in the Aucklands. The Chathams have a total flora of 152, of which 27 occur nowhere else, leaving 125, of which 30 occur in the Kermadecs, and 32 in the Aucklands. The Aucklands have a total flora of 119, of which 44 do not occur in New Zealand, leaving 75, of which 32 occur in the Chathams and 5 in the Kermadecs.

(15) We may predict that the species in common between the islands, or between the islands and Stewart, will show a greater proportion of wides (which on the whole are older than endemics), the more the islands on which the species occur.

TABLE X.

	Wides.	*Proportion.*	*Endemics.*	*Proportion.*
3 islands and Stewart	4	80 %	1	20 %
2 „ „ „	27	64 %	15	36 %
1 „ „ „	43	41 %	61	59 %
No „ but „	57	24 %	174	76 %
Not even reaching Stewart	170	18 %	749	82 %

The proportions decrease and increase, respectively, with regularity.

(16) We may now go on to some slightly more general predictions, and say that as on the whole the islands were probably cut off earlier than Stewart, their plants will on the whole be older, and should therefore show a greater proportion of families to genera and genera to species.

TABLE XI.

	Families.	*Genera.*	*Gen. per fam.*	*Species.*	*Spp. per gen.*
Islands	55	130	2·3	199	1·5
Stewart	60	169	2·8	382	2·2
New Zealand	91	329	3·6	1392	4·2

(17) We may make the same prediction with regard to the islands as the second made with respect to Stewart on p. 34 of 11, that on the whole

the more genera in a family the older the family will be, and therefore the better represented.

TABLE XII.

Fam. repres. in N.Z. by	*In N.Z.*	*In Stewart.*	*In islands.*	*Not there.*
1 genus	36	16	15 41 %	21
2 genera	15	7	10 66 %	5
3 ,,	15	13	10 66 %	5
4–5 ,,	10	10	10 100 %	—
6–10 ,,	9	8	8 90 %	1
over 10 ,,	6	6	6 100 %	—

(18) Similarly we may repeat the prediction (3) on the same page, and say that the same thing will be true with regard to the genera. This also proves to be the case, the proportions of genera that occur being 21, 35, 58, 55, 66, 68, and 80 per cent., a progressive table, save for the one slight drop from 58 to 55 in the middle.

(19) We may go on to predict that the plants reaching 3 groups of islands, New Zealand, and Stewart, 2 groups, 1 group, Stewart only, and the main islands of New Zealand only, should show a progressive decrease in the average size of families and genera.

TABLE XIII.

Reaching	*Aver. size of fam.*	*Aver. size of genus.*
3 islands	88	6[1]
2 ,,	42	13
1 ,,	29	7·8
Stewart only	18·5	6·8
N.Z. as a whole	15	4·2
N.Z. only	2·9	1·5

(20) All the predictions we have made with regard to the flowering plants should also hold in the case of the ferns, and a very cursory examination shows that they do, so that it would be a work of supererogation to go into details; but we may predict that as the ferns are on the whole an older group than the flowering plants, those of the flora of Stewart will be on the whole better represented. Examination shows that no less than 64 per cent. of the ferns of Stewart are represented on the islands, against only 40 per cent. of the species of flowering plants.

There are other predictions that might be made with regard to families, genera, and species, but we must go on to other more general features, and may begin with the species endemic to the islands only, which are fairly numerous.

(21) Upon the hypothesis of Natural Selection one might expect that some of these endemics at any rate would be relicts. If this were so, one might surely expect to find some of them occurring upon two of the groups of islands and not in New Zealand; they might have spread to these

[1] Only 5 genera with 30 species, too small a number for a reliable result.

islands in early times, and have since been killed out in the more crowded main islands of New Zealand. But in actual fact we find that there are none with such a distribution; all that occur in the Kermadecs and Chathams, or Chathams and Aucklands, occur also in New Zealand, as would be expected on the hypothesis of age and area. There are a good many which occur in the Aucklands and some of the other islands in the same stretch of sea (Campbells, Antipodes, and Macquarie), but none that occur in two of the main groups of islands and not also in New Zealand.

(22) One will expect the endemics to belong on the whole to the oldest, i.e. in general to the largest, families and genera in New Zealand. They belong in fact to Ranunculaceae, Caryophyllaceae, Geraniaceae, Rosaceae, Umbelliferae, Cornaceae, Rubiaceae, Compositae, Epacridaceae, Myrsinaceae, Gentianaceae, Boraginaceae, Scrophulariaceae, Plantaginaceae, Chloranthaceae, Urticaceae, Liliaceae, Juncaceae, Palmae, Cyperaceae, and Gramineae, 21 families, which contain in New Zealand and the islands as a whole 939 species, or 44 species per family, whilst no endemics occur in the other 70 families of the New Zealand flora, with 453 species (average 6·4 per family), except in the genera *Scaevola*, *Homalanthus*, and *Boehmeria*, which occur in the Kermadecs, but not in New Zealand.

Grouping all the families of New Zealand in order of size, we find that 52 endemics occur in 8 of the first 10 families, 10 in 5 of the second 10, and 3 in the other 2 families which are above the average size, or 65 endemics in all in families which are above the average in size, whilst 8 only occur in all the 68 other families which are below the average in point of size.

In the same way, 60 endemics belong to 25 genera which are above the average, and 13 to 11 genera which are below it.

(23) Being, on our supposition, very old genera, all the genera which contain endemics confined to the islands, unless possibly some of the later ones of the invasions, should occur in Stewart. Three genera occur in the Kermadecs only, and are not found in New Zealand, so that they may be looked upon as genera which arrived in those islands from the north too late to reach New Zealand, and three are endemic genera of the islands. All the remaining genera, except *Corokia* and *Rostkovia*, that contain island endemics, occur in Stewart. *Corokia* is a distinctly northern type, and the endemic occurs in the Chathams; *Rostkovia* probably a late arriving southern type, whose endemic occurs in Campbell.

It is thus clear that, just as in Stewart Island, the endemics of the islands belong chiefly to the 'successful' families and genera of New Zealand. The families are given above, and the actual genera are *Ranunculus* (4 endemics), *Stellaria*. *Colobanthus*, *Geranium*, *Geum*, *Azorella*, *Aciphylla* (2), *Ligusticum* (3), *STILBOCARPA*, *Pseudopanax*, *Corokia*, *Coprosma* (4), *Olearia* (4), *PLEUROPHYLLUM* (3), *Celmisia* (2), *Cotula* (3), *Abrotanella* (2), *Senecio* (2), *Sonchus*, *Cyathodes*, *Dracophyllum*, *Myrsine* (3), *Gentiana* (5),

MYOSOTIDIUM, *Veronica* (4), *Plantago*, *Ascarina*, *Urtica*, *Bulbinella*, *Rostkovia*, *Carex* (2), *Imperata*, *Hierochloe*, *Deschampsia* (2), *Poa* (7), *Festuca* (2). Those which have no number against them are represented by one species only, and those in capitals are endemic genera of the islands only. No one can look over this list of genera, and honestly suggest that it represents a set of relicts. Many are genera of world-wide distribution, and all are common, except the three endemic genera, with 5 species, which represent most of the species which might perhaps be looked upon as relicts.

(24) We have seen (10, pp. 358, 361) that the northern invasion of New Zealand was nearly all tree-like, the southern herbaceous. We shall therefore expect the proportion of trees to be greatest in the endemics of the northern islands, of herbs greatest in the southern. In actual fact, the Kermadecs have 7 endemic shrubs and trees and 2 herbs (both Monocotyledons), the Chathams have 13 shrubs and trees and 12 herbs (4 Monocotyledons), and the Aucklands 3 shrubs and trees only against 27 herbs (7 Monocotyledons), whilst in the Campbells there are 3 shrubs, in the Antipodes 1, and in Macquarie none. This bears out the prophecy completely, and it may also be pointed out that the three endemic genera of the islands are composed entirely of herbs (*Stilbocarpa*, *Pleurophyllum*, *Myosotidium*), a fact which does not harmonize well with Prof. Sinnott's suggestion that endemics are relicts and herbs the youngest forms.

It is clear, from the success of the 24 predictions above given, that, just as in the case of Stewart Island, it is hardly conceivable that the outlying islands should have received their floras by casual transport across the intervening seas. There must have been connexion by land at some time. When one works out their species one by one, it is fairly clear that a few—at most hardly exceeding, if they even equal, 10 per cent.—may have been carried by water or by birds, but the great bulk must undoubtedly have arrived by land. In no other way could the great similarities between the islands, or between the islands and Stewart, have come about. No casual transport could give results like those above, nor would it occur that the island endemics belonged almost solely to the larger families.

The Kermadec Islands.

We must now go on to consider in brief some of the relations of the islands to one another, and to New Zealand and its probable invasions of plants (10, p. 355). Let us begin with the Kermadecs. They have altogether, according to Cheeseman, a flora of 71 species, made up thus:

Table XIV.

Dicotyledons	31 families	47 genera	56 species
Monocotyledons	6 „	15 „	15 „

Of these 62 genera and 71 species, there do not occur in New Zealand at all, in addition to the local endemics of the islands (belonging to genera of New Zealand), no less than 8 genera and 8 species, viz. *Canavalia obtusifolia* (cosmotropical), *Ageratum conyzoides* (cosmotropical), *Scaevola gracilis* (Kermadecs only), *Aleurites moluccana* (trop. Asia), *Homalanthus polyandrus* (Kermadecs only), *Boehmeria dealbata* (ditto), *Panicum sanguinale* (cosmotropical), *Cenchrus calyculatus* (Polynesia). Besides these there occur in these islands *Metrosideros villosa* (Polynesia), *Coprosma petiolata* and *acutifolia* (endemic), *Myrsine kermadecensis* (ditto), *Ipomoea biloba* (cosmotropical), *Ascarina lanceolata* (endemic), *Cordyline terminalis* (Polynesia, Indomalaya), *Rhopalostylis Baueri* (Norfolk I.), *Imperata Cheesemanii* (endemic), *Eleusine indica* (trop. Asia), and *Poa polyphylla* (endemic), 10 genera in all with 11 species, the genera, but not the species, occurring in New Zealand.

Now as these species, which do not occur in New Zealand proper, are either endemic to the Kermadecs, or common to the Kermadecs and Polynesia, it would seem probable that the Kermadec Islands received part of their flora not by way of New Zealand, but by way of the ridge which runs north from these islands to Tonga and Fiji, or at any rate from some part of Polynesia.

Subtracting these from the total of the Kermadec flora leaves only 52 species found there which actually occur in New Zealand, and it is of interest to trace out their distribution there. Twenty-five belong to Class 1 in order of rarity, ranging New Zealand (including Stewart) from end to end, and of these no less than 20 reach the Chathams also, and 5 the Aucklands as well. It may be noted that the 5 which have not been recorded from the Chathams are *Melicytus ramiflorus* (tree), *Haloragis alata*, *Tetragonia expansa*, *Apium prostratum*, and *Agropyrum scabrum* (herbs). A further 13 belong to Class 2, ranging from North Cape to Foveaux Strait, and of these only 6 reach the Chathams, a much smaller proportion, corresponding to the generally lesser age. With regard to these species, 38 in all, it seems to me almost impossible at present to make any safe deductions as to whether they reached New Zealand by way of the Kermadecs, or vice versa.

There remain 14 species with less range, all of which range northwards to North Cape. Several of these belong to genera which are otherwise unrepresented in New Zealand, e. g. *Piper excelsum* (Polynesia, Class 3) *Corynocarpus laevigata* (K. and N. Z. only, Class 4), *Oplismenus undulatifolius* (K. and N.Z. only, Class 4), *Sicyos angulata* (Polynesia, Class 5), *Acianthus Sinclairii* (K. and N. Z. only, genus New Caled., Class 5), *Rhagodia nutans* (Australia, Class 6), *Siegesbeckia orientalis* and *Bidens pilosa* (palaeotropical, Class 7), *Peperomia Endlicheri* (Lord Howe Island, Norfolk Island, Class 7). These species it would seem justifiable to regard as having entered New Zealand by way of the Kermadecs.

There remains one species in Class 9, *Ipomoea palmata*, which ranges about 80 miles from North Cape. The only other species of *Ipomoea* in this region, *I. biloba*, occurs only in the Kermadecs. It is fairly clear from the very short range that *I. palmata* probably did not reach New Zealand from the Kermadecs by land. It is more probable that it has only comparatively lately, so to speak, arrived, and by water carriage. There are hundreds of miles of the northern coast on which it would in all probability be equally at home.

It is thus fairly evident that while a large part of the flora of the Kermadecs was in all probability derived from New Zealand, it is at least extremely probable that some of it was derived directly from some part of Polynesia, probably by way of the Tonga-Fiji ridge. This supposition is confirmed by the behaviour of the ferns, already pointed out (9. p. 341). A number of Polynesian ferns appear to have entered New Zealand by way of the Kermadecs, and in New Zealand only range part of the length of the islands.

When we come to compare the Kermadec flora with that comprised in the northern invasion (10, p. 356), and find that 22 of the 33 families are missing, it seems hardly possible, in view of what we now know about the rarity of dying out among flowering plants at any rate, that the northern invasion passed by way of the Kermadecs. On the other hand, we have seen that the only members of Anacardiaceae, Cucurbitaceae, and Piperaceae, three of the 33 families enumerated in it, probably passed to New Zealand by way of the Kermadecs. It would seem therefore probable that these three families should be excluded from the list of the northern invasion proper, and should be classed, together with the other plants mentioned above (*Siegesbeckia*, &c.), as a second northern (Kermadec) invasion.

From the extreme regularity of the distribution of the Kermadec plants in New Zealand (except the conspicuously different *Ipomoea palmata*), one is obliged, it seems to me, to conclude that there was at one time a land bridge connecting the two.

We also know (10, p. 358) that the northern invasion was mainly (84 per cent.) trees and shrubs. Examination of the Kermadec flora, however, shows that it contains 45 herbs and only 26 shrubs and trees. The latter thus form only 36 per cent. of the flora, a very different proportion from that in the northern invasion proper.

Of the 26 shrubs and trees, 12 are confined to the Kermadecs, or at least do not reach New Zealand, and 5 of the other 14 range from end to end of New Zealand, the mean range of the whole 14 being represented by the figure 3·0. Of the 45 herbs, 7 only are not found in New Zealand, 20 range New Zealand from end to end, and the mean range is represented by the figure 2·2. These facts are inclined to suggest, though they offer no

proof, that herbs may range more rapidly than trees and shrubs, as indeed one is inclined to think must necessarily be the case. Eight of the 14 shrubs and trees reach the Chathams also, and only 17 of the 38 herbs, a fact which also points in the same direction, as it gives one to suppose that the former were opposite to the Chatham connexion at an earlier period than the latter.

(25) Finally, many Kermadec species also reach the Chathams, as we have seen. Now if age be the chief determinant in distribution, we shall expect (cf. map-diagram 2) that those of these forms which belong to the chief northern invasion, supposed to centre in Auckland, will also reach Dunedin to the south, while those which do not may cease at a less distance from the North Cape. Examining the facts, we find that of the 29 species which the Kermadecs have in common with the Chathams, 26 range to Dunedin or farther south, whilst *Piper excelsum* reaches only to Banks Peninsula and Okarito, *Corynocarpus laevigatus* to Banks Peninsula and Westland, and *Acianthus Sinclairii* to Dun Mountain and Westport. All of these, we have seen above, may very probably be regarded as having entered New Zealand in the Kermadec invasion; and it is also noteworthy that *Corynocarpus* is a tree, which might have been early enough to reach the Chathams without perhaps travelling sufficiently fast to have reached so far south as some of the other forms also common to the Kermadecs and Chathams. There are 16 other Kermadec species which reach as far south as *Corynocarpus* and do not reach the Chathams; 13 of these are herbs, *Melicytus ramiflorus* and *Panax arboreum* are trees, and *Coprosma Baueri* is a shrub.

The Auckland Islands.

Turning now to the southern end of New Zealand for a while, let us look at the flora of the Aucklands and their surrounding islands (the Antipodes, Campbell, and Macquarie). The first thing that strikes one is that though the Aucklands are subantarctic and the Kermadecs subtropical, the former have a much larger flora recorded, 119 species against 71. Of these 44 do not occur in New Zealand proper.

As regards constitutional habit, the Aucklands contain only one tree (*Metrosideros lucida*), 11 shrubs, and 107 herbs. The last named thus form 90 per cent. of the total, against only 64 per cent. in the Kermadecs.

Another feature of interest is the great proportion of Monocotyledons. To deal properly with this feature, we must briefly consider the distribution of Monocotyledons in New Zealand itself, though complete details must be left for subsequent papers. If we divide the main islands of New Zealand from north to south into zones of 100 miles, as has already been done upon several occasions (cf. 6, p. 444), and tabulate the species that occur in them, we get for the Monocotyledons:

TABLE XV.

	-100 m.	-200	-300	-400	-500	-600	-700	-800	-900	-1000	-1080
Wides reaching islands	21	21	23	23	24	26	26	26	26	29	26
Wides (N.Z. only)	71	68	85	84	83	80	76	69	62	56	27
Endemic N.Z. and islands	22	22	23	25	27	31	31	30	31	31	30
Endemic N.Z. only	56	63	71	81	80	100	98	98	104	86	39
	170	174	202	213	214	237	231	223	223	202	122

It is clear that the centre of the Monocotyledons as a whole is in the South Island. But the numbers do not run with perfect regularity to a maximum at one zone, and this leads one to suspect, what indeed seems probable when one realizes (10, p. 355 seq.) that there have been at least two invasions of New Zealand, that the group really entered partly in one, partly in the other, invasion. The southern invasion of Monocotyledons was, as we shall see, so much the larger that its figures all but swamp those of the northern.

If we now examine the Monocotyledons genus by genus, we soon find that they divide into two main groups, one commencing at the north end of New Zealand and ranging to a greater or less distance south, the other commencing at the south end and ranging to a greater or less distance north. It is very striking how few species there are which have intermediate ranges, among the wides—the endemics of course have every possible range. The only wides that do not reach one or the other end of the two main islands of New Zealand are given in the table below (I have counted those wides that only reach the southern end of the narrow peninsula at the extreme north as reaching the north end of New Zealand, this peninsula being so entirely different from the rest of the country, and so small in area).

TABLE XVI.

Prasophyllum rufum
Pterostylis mutica
Hypoxis pusilla
Juncus scheuchzerioides
Triglochin palustre
Potamogeton palustre
Zannichellia palustris
Lepilaena Preissii
Centrolepis strigosa
Lepyrodia Traversii
Eleocharis acicularis
Uncinia Sinclairii
tenella
Carex acicularis
lagopina
leporina
Brownii
Imperata arundinacea
Stipa setacea
Alopecurus geniculatus
Agrostis parviflora

A mere glance at this list, for any one who is familiar with Cheeseman's Flora, is sufficient to show that it includes, as is usual in lists of species that behave irregularly (as regarded from the point of view of age and area), the bulk of the doubtful determinations, species of possible recent introduction, &c. The three orchids might possibly have been brought by the aid of the wind in comparatively recent times. There are only perhaps 8 of the 21

which can be regarded as probably in reality ranging short of either end of New Zealand, and which are not either wrongly named or probably introduced, whilst it is obvious that any further discoveries of these species in new localities will go to make the original contention, that the wides in general range to one or the other end of New Zealand, nearer and nearer to the exact truth. In any case, as there are 179 Monocotyledon wides, it is clear that an 'error' of 8 is nothing very marked.

Beginning with the orchids, we may probably regard as northern genera (inasmuch as their wides, where they do not range completely along New Zealand, usually begin at the north end) *Dendrobium*, *Bulbophyllum*, *Earina*, *Sarcochilus*, *Spiranthes*, *Thelymitra*, *Orthoceras*, *Microtis*, *Caleana*, *Acianthus*, *Cyrtostylis*, *Calochilus*, and *Gastrodia*. Similarly we may regard *Cordyline* and *Astelia* in Liliaceae, *Rhopalostylis* in Palmae, *Freycinetia* in Pandanaceae, *Kyllinga*, *Cyperus*, *Mariscus*, *Fimbristylis*, *Scirpus*, *Schoenus*, *Cladium*, *Lepidosperma*, and *Gahnia* in Cyperaceae, and *Imperata*, *Zoysia*, *Paspalum*, *Isachne*, *Oplismenus*, *Spinifex*, *Sporobolus*, *Dichelachne*, *Amphibromus*, *Bromus*, and *Agropyrum* in Gramineae, as northern types. If now we take the zonal distribution of the species of these genera, and add them up, we get:

TABLE XVII.

	-100 m.	-200	-300	-400	-500	-600	-700	-800	-900	-1000	-1080
Wides reaching islands	14	14	14	14	14	15	15	13	13	13	10
Wides (N.Z. only)	36	32	32	30	25	21	17	11	5	3	3
Endemic N.Z. and islands	13	13	13	14	14	14	14	12	12	12	11
Endemic N.Z. only	28	27	29	31	24	23	19	16	14	10	6
	91	86	88	89	77	73	65	52	44	38	30

tapering markedly from north to south. If now we subtract this table from Table XV, we get:

TABLE XVIII.

	-100 m.	-200	-300	-400	-500	-600	-700	-800	-900	-1000	-1080
Wides reaching islands	7	7	9	9	10	11	11	13	13	16	16
Wides (N.Z. only)	35	36	53	54	58	59	59	58	57	53	24
Endemic N.Z. and islands	9	9	10	11	13	17	17	18	19	19	19
Endemic N.Z. only	28	36	42	50	56	77	79	82	90	76	33
	79	88	114	124	137	164	166	171	179	164	92

These figures clearly show that the supposition of two chief invasions of Monocotyledons is supported by the facts; and it is probable that when our detailed knowledge of distribution in New Zealand reaches comparative perfection, we may be able to go into even greater detail. As it is, one or two genera, e.g. *Scirpus* or *Schoenus*, have wides beginning at both the north and the south ends of New Zealand, and ranging part way along the islands to the south or to the north. If these genera were regarded as having entered by

both invasions, and their species divided between them, the slight irregularities shown in the tables above would be removed.

(26) The southern invasion of Monocotyledons being thus much larger than the northern, we shall expect the Aucklands to show a larger percentage of this group in their flora than do the Kermadecs, and we shall expect the Chathams to show an intermediate percentage.

TABLE XIX.

	Aucklands.		*Chathams.*		*Kermadecs.*	
Monocotyledons	54	45 %	49	31 %	15	21 %
Dicotyledons	65	55 %	106	69 %	56	79 %

(27) It follows incidentally from what has been said that Stewart Island should show a greater proportion of Monocotyledons in its flora than New Zealand as a whole. This comes out in

TABLE XX.

	New Zealand as a whole (including islands).		*Stewart.*	
Monocotyledons	348	27 %	130	34 %
Dicotyledons	954	73 %	252	66 %

or, to put it in another way, 37 per cent of the New Zealand Monocotyledon flora occur in Stewart, and only 26 per cent. of the Dicotyledon flora (neglecting in each case the few Stewart endemics). The whole makes a very awkward problem for the supporter of Natural Selection; why are Monocotyledons so much better suited to Stewart and the Aucklands than to the Kermadecs, and why are the Chathams intermediate in the proportion they bear? But if we simply recognize that the proportions are merely due to the size and time of the invasions of New Zealand by plants, we get a perfectly simple and straightforward explanation.

(28) Examination of map-diagram 2 will show that if we draw a circle with its centre in the Auckland Islands, passing through the Chathams, it will also pass through Auckland City. We shall therefore expect that in general (unless of course there was not direct land communication in either of these directions), species which reach both the Aucklands and the Chathams will also reach Auckland City. Examination of the facts shows that of the 32 species that these groups of islands have in common, 26 are found at least as far north in New Zealand as Auckland City. Of the other 6, 2 are wides, both South American species, *Tillaea moschata* and *Veronica elliptica*, reaching, the one to the northern side of Cook's Strait, the other to West Wanganui and Cape Foulwind, in South Island. *Coprosma foetidissima* reaches to the Thames goldfields, and *Deschampsia caespitosa* to the lower Waikato, so that these two reach within a very short distance of Auckland. *Tillaea moschata* and *Rumex neglectus* are

coast species, which may have reached the Chathams by water, and *Urtica australis* finally, which only reaches the small islands in Foveaux Strait, is also probably a water-carried species. The prediction is thus borne out by the facts as well as can be expected. Even if all the six species be considered as exceptional, the prediction is within 20 per cent. of accuracy.

Both the southern invasion, and the floras of the Aucklands, Campbell, &c., contain a large number of South American forms, and the question has been much discussed as to how they have reached New Zealand, or whether indeed they did not reach South America from New Zealand. Guppy (5, p. 294) discusses the possibilities of water carriage, and shows that seeds drifted from South America could only reach the extreme north of New Zealand. But an examination of the New Zealand forms with South American affinities shows that those which do not range the entire length of the islands are mainly concentrated in the south. The only ones with northern location are *Sicyos angulata* (range 0–540), *Mesembryanthemum aequilaterale* (360–500), *Gratiola peruviana* (0–940), *Cyperus vegetus* (60–500), and *Scirpus sulcatus* (80–700, Tristan da Cunha, not South America proper). Of these we have seen that the first probably entered from the Kermadecs, it also occurring in Polynesia and Australia, and the last may have been drifted by water from Tristan. *Mesembryanthemum* and *Gratiola* also occur in Australia, so that there remains only *Cyperus vegetus*, and it is permissible to suppose that this may have arrived by water carriage, perhaps when the Lord Howe I. bank was dry land.

Water carriage, however, will hardly explain how the others reached New Zealand from South America, and there remains the possibility that the transport was the other way, and that these species reached South America from New Zealand. Guppy shows that transport is possible from the south of New Zealand to Chili. The genera involved are mostly so cosmopolitan that one cannot argue from the generic distribution, and must take other matters into consideration. The great argument against this supposition, to my mind, is based upon age and area. These species, in South America, mostly reach not only Chili, but also Fuegia, the Falkland Islands, South Georgia, &c., and in some cases also Kerguelen and other islands of the Antarctic Ocean. It is, therefore, clear that they must be enormously old in South America, and if they had gone there from New Zealand they must be yet older in that island, and should therefore be very widely distributed there, which is exactly what does not occur. Their distribution shows clearly that they must be older in South America, and must therefore have gone *to*, not from, New Zealand.

Or again, we may take single examples. It would seem very strange if *Ranunculus biternatus* spread from Macquarie to South America (it does not occur in New Zealand), or *Cardamine glacialis*, found only in the Aucklands, Campbell, Macquarie, and South America, or other species

found only in limited areas in the South Island. The South American wides are no commoner, in fact slightly rarer (less distribution area) in New Zealand than the Australian wides, and it may also be noticed that those South American wides that also reach Australia are very widespread indeed in New Zealand.

There seems therefore some reason to suppose that the affinity between New Zealand and South America is really due to the fact that at one time there was land connexion, complete or nearly complete, between them, though a few species, especially those with northern range in New Zealand, may owe their presence to water carriage. The equatorial current from the west coast of South America passes by way of the Kermadec and Chatham Islands.

As to the position and direction of the land bridge, it is very difficult to come to any definite conclusion in the present state of our knowledge. In general we may perhaps imagine that it went by way of the Antarctic continent, perhaps all the way to South Georgia, or perhaps by way of Juan Fernandez to Chili, though this seems unlikely. But a land connexion seems to me to be indicated by the facts of distribution.

As so many Australian and Tasmanian forms are included in the plants of the southern invasion, it would seem probable that this land connexion extended also to Australia and Tasmania.

Whether the South American and the Australian plants arrived by the same route from the south it is difficult to say. The general facts seem to me to indicate—it is impossible to put it into words without a vast amount of detail—that it is quite possible, if not probable, that the land joining New Zealand to the Antarctic continent was more or less broken up by water areas. It is, for example, noticeable that a number of species which occur in Kerguelen, the Crozets, Marion Island, &c., are found only in Macquarie, though of course this is quite possibly due to water carriage.

The next question is, Did this connexion with Antarctica pass through any of the groups of islands that we are now considering? As even the Aucklands only possess 39 out of 127 wides of the southern invasion, and Campbell, the Antipodes, and Macquarie progressively less, it may be inferred that it did not pass directly through any of them, and that it was probably nearest to the Aucklands.

The average rarity in New Zealand of the Auckland wides as a whole is 2·7, while that of the wides of the southern invasion is 3·1. This is little to go upon, but at least it tends to show that on the whole the arrivals of wides in the Aucklands were fairly early.

There are a considerable number of South American genera which occur in New Zealand but not in the Aucklands, another fact which goes to show that the southern invasion, or some of the invasions, if there were more than one, did not pass directly through these islands.

In Campbell, the Antipodes, and Macquarie we find in general the Auckland flora, in less and less proportion, so that on the whole these islands must probably have received their floras by the same invasion. It would lead too far to go into greater detail.

The Chatham Islands.

To turn lastly to the Chathams, it is obvious that they could never have formed part of a bridge from New Zealand to anywhere else, the water on their eastern side being almost the deepest in the world, so that their flora, except in so far as it may have been brought by currents, must have come through New Zealand. Even if the whole area shown in the map as above the depth of 1,000 fathoms were dry land, a species beginning between the Chathams and Antipodes would as a rule have also reached New Zealand by the time that it had reached both groups of islands.

The Chathams are fairly opposite to the middle of New Zealand, and connected by shallower water with the South than with the North Island, so that one will expect their flora to be fairly rich, unless they were cut off very early indeed, a matter as to which we have no information. As a matter of fact they have the richest flora of the outlying island groups, composed of 106 species of Dicotyledons and 49 of Monocotyledons, the proportion of the latter being, as we have seen, intermediate between that in the Kermadecs and that in the Aucklands.

(29) We shall expect that on the whole the families missing in the Chathams from the Stewart list, which is likewise old, will be the smaller families of that island, i. e. on the whole those which have been the latest in arriving there. If we omit all those with one species, we omit Magnoliaceae, Pittosporaceae, Elatinaceae, Hypericaceae, Linaceae, Cornaceae, Goodeniaceae, Primulaceae, Apocynaceae, Loganiaceae, Labiatae, Illecebraceae, Lentibulariaceae, Chloranthaceae, Loranthaceae, and Euphorbiaceae. Primulaceae must certainly occur in the Chathams, as the only species in the family occurs in both Kermadecs and Aucklands. Leaving this out, the only other families in this list which are recorded from the Chathams are Linaceae, Cornaceae, Labiatae, and Euphorbiaceae. The other Stewart families not recorded from the Chathams are Portulacaceae (2 species), Tiliaceae (4), Saxifragaceae (3), Droseraceae (4), Stylidiaceae (4), Ericaceae (2), Plantaginaceae (3), and Centrolepidaceae (2), but we have already seen that (prediction 5) we must expect the Chathams not to contain the very small families of the southern invasion. This excludes from this list Portulacaceae, Droseraceae, Stylidiaceae, Plantaginaceae, and Centrolepidaceae, leaving only three families of the Stewart flora that might be expected and have not been recorded, and four recorded that would hardly be expected, as being families with only one species in Stewart. The seven families that occur in the Chathams and do not as yet appear to have been recorded

from Stewart, Rhamnaceae, Anacardiaceae, Leguminosae, Solanaceae, Myoporaceae, Piperaceae, and Palmaceae, are all northern except perhaps Leguminosae, which is the largest New Zealand family not yet recorded from Stewart.

(30) In the same way, we shall expect the smaller genera of the Stewart flora to be those that are chiefly missing, and on examination we find that of those represented in Stewart by one species 57 are not found in the Chathams, and 31 have been found, while of those represented by two or three species 19 have not been found, and 26 are present, in the Chathams.

(31) One may, by the aid of age and area, go a good way towards a prediction of the whole flora of the Chathams (cf. that of the flora of Stewart in 11, p. 27). If one first predict that they will contain the Kermadec species that reach Dunedin, this gives 38 species of which 26 reach the Chathams; then adding that they will contain the Auckland species that reach Auckland city gives about as many more. If now one add to this that the bulk of the rest of the flora will likely be made up from the species which range the whole length of New Zealand (for those that ranged less distance would as a rule be too young to reach the Chathams at all, unless they ranged beyond New Zealand to the Kermadecs or Aucklands), one obtains almost all the remaining flora, except the local endemics, which we have already seen may be to some extent predicted as likely to belong to the oldest families in the Chathams, that is to say, to the families represented by the most species in New Zealand (see above, p. 270). Of course this prediction that the Chatham flora will be selected almost entirely from Classes 1 and 2 (6, p. 449) of the New Zealand flora brings in also a great many other species belonging to those classes, but which do not occur in the Chathams. We have as yet no means of deciding which are the oldest species in a given class, and it will only be the oldest, in general, which will reach the Chathams. The fact remains, however, that by this prediction we cover all of the Chatham flora but 14 species and the local endemics, and we have seen that we can more or less closely predict the families and genera to which these belong. These unpredicted species are:

Pomaderris apetala: a special case, see 8, p. 332.
Corynocarpus laevigatus: see above, p. 279.
Tillaea moschata: a coast species.
Epilobium insulare: lowland swamps.
Coprosma foetidissima: see above, p. 284.
Helichrysum filicaule: dry grassy places.
Veronica elliptica: a coast species.
Piper excelsum: usually near the coast.
Urtica australis.
Pterostylis australis: cf. 11, p. 39.

Acianthus Sinclairii.
Rhopalostylis sapida.
Lepyrodia Traversii: doubtful determination, see Cheeseman.
Deschampsia caespitosa: see above, p. 284.

Thus several are doubtful determinations, or coast forms, which may have been brought by the currents. But it is only among these few species that one can look for exceptions to age and area; all the remainder of the flora of the Chathams—155 species in all—is easily explained by the simple operation of this law, and no other assistance is needed to explain it.

(32) We may predict that the Chathams should have proportionately more species in the higher classes of width of distribution than the Kermadecs or Aucklands, where more recent arrivals may occur, as these islands have been nearer to the tracks of the invasions.

TABLE XXI.

The Chathams have	155	species, of which	77	are Class 1,	30	Class 2
,, Kermadecs ,,	71	,,	,,	22	,,	,,	16	,,
,, Aucklands ,,	119	,,	,,	35	,,	,,	6	,,

The proportion (and the totals) in the Chathams is by far the greatest.

One might give other predictions about the floras of these islands, but these will suffice to show that in a well-defined area like New Zealand and its surrounding islands one can without hesitation draw upon the hypothesis of age and area to make predictions about the taxonomic distribution of the flora, and find that the predictions are justified by the facts. I have now used the hypothesis to make no fewer than 67 predictions (32 in this paper), every one of which has proved to be correct. In several cases the exactness with which the prediction has been borne out by the facts has been positively astonishing, and in all cases the result has been as near to accuracy as can reasonably be expected in a biological subject, and especially in one so complex as geographical distribution, where changes in the configuration of the land and sea may be continually going on, and in a very complicated way. It seems to me, therefore, that the hypothesis of age and area, having been successfully used to make so many predictions, may now perhaps be regarded as being fairly well established as one of the chief (if not the chief) positive factors in geographical distribution, while the action of barriers, which may be of many kinds—seas, rivers, mountains, ecological barriers, changes of climate, &c.—may be regarded as the chief negative factor, and other things, such as the action of man, are also of very great importance indeed.

If this position be regarded as reasonable, it is evident that we must now state the hypothesis in rather more clear and definite terms than those in which it was first formulated, e.g. in 6, p. 438. After careful

consideration of the various papers that have been written on the subject, and after devoting much time to further work upon it, I am inclined to word the hypothesis thus:

The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climate from one region to the next or other ecological boundaries, and the like, also by the action of man, and by other causes.

In other words, age and area is the chief positive, the action of barriers the chief negative, factor in plant distribution, while in recent times the action of man has become of greater importance than either.

It is clear that in general this law also covers the case of a genus of more than a very few species, for a genus is in general a group of allied species, though it is becoming every year more clear (cf. 1 and 12, p. 446) that many genera are based upon too few characters, and are in reality polyphyletic.

The acceptance of this hypothesis will involve various changes in our methods of handling problems of geographical distribution, and in further papers I shall go on to indicate some of these.

Summary.

In this paper the chief attention is devoted to the islands outlying around New Zealand, especially the Kermadecs, Chathams, and Aucklands, and the following points are indicated, chiefly by the method of prediction and subsequent verification, which is here used successfully no fewer than 32 times. It is first shown that the floras of these islands must be very old, from their far outlying position, and is then indicated that—

1.[1] The islands have proportionately more families in common with Stewart, whose flora is also old, than with New Zealand proper (Table I).

2. The great proportion of the families in the different islands are the same, and most are selected from the Stewart list (Table II).

3. The families reaching three groups of islands are on the whole the largest (oldest), those reaching two next, and then those reaching one or none (Table III). The same is true for the Stewart families (Table IV), and the total result is summed up in Table V.

4. The Stewart families that are missing in the islands are on the whole the smallest.

5. The Aucklands and the Chathams contain most of the families of the southern invasion, and those that are missing in the Chathams are the smallest, numerically, of that invasion.

[1] Predictions numbered as in text above.

6. The Chathams contain many families of the northern invasion.

7. The outlying islands have proportionately more families in common with one another than they have with New Zealand (Table VI).

8. The genera of the floras of the outlying islands are chiefly selected from the Stewart list (Table VII); only 26 out of 140 do not occur there, and are mostly northern genera.

9. The genera that occur in the islands are on the whole the largest in number of species, both in Stewart and New Zealand (Table VIII).

10. The islands have proportionately more genera in common with one another than they have with New Zealand (Table IX).

11. The Stewart genera which are missing are on the whole the smallest.

12. The species in the islands are also to a very large extent indeed selected from the Stewart list.

13. The Stewart families that have no species in the islands are on the whole very small in Stewart.

14. The islands have very many species in common among themselves.

15. The species in common between three islands (the oldest) show the largest proportion of wides (the oldest forms), then those of two islands, one island, and those reaching none (Table X).

16. The plants of the islands show a greater proportion of families to genera and genera to species than those of Stewart and New Zealand (Table XI).

17. The more genera in a family in New Zealand, the older on the whole is the family in New Zealand, and the better represented in the islands (Table XII).

18. The same thing shows in regard to the genera.

19. The plants reaching 3 groups of islands, New Zealand and Stewart, 2, 1, Stewart and New Zealand only, and New Zealand only, show a progressive decrease in the average size of families and genera (Table XIII).

20. All these predictions are also true of the ferns, and the fern flora of Stewart, as on the whole older, is better represented in the islands than the flowering plants.

21. The species endemic to the islands only do not occur on two of the chief groups without occurring in New Zealand, as one might expect were they relicts.

22. The endemics of the islands belong on the whole to the largest (i.e. on the whole the oldest) families of New Zealand.

23. Practically all the genera of the island endemics, being old, occur in Stewart.

Just as in Stewart, the island endemics belong mainly to the 'successful' families and genera of New Zealand.

24. The proportion of trees is greatest in the northern islands, least in the southern, intermediate in the Chathams.

The islands must have had connexion by land with New Zealand.

The Kermadecs contain a number of species which do not occur in New Zealand, but occur in Polynesia; it therefore seems probable that they received part of their flora direct from Polynesia.

The distribution of the Kermadec species in New Zealand is considered, and it is shown that it does not agree with any hypothesis but that of a land bridge between the two.

The Kermadecs probably were not in the track of the main northern invasion of New Zealand, but were upon the route of a minor invasion from Polynesia.

25. The Kermadec species which reach the Chathams also reach in general as far south in New Zealand as Dunedin.

The Aucklands show a great proportion of Monocotyledons, and the distribution of this group in New Zealand is briefly considered, it being shown that it probably took part in both invasions.

The wides of New Zealand nearly all range to one or the other end of the islands, if not to both. Those which do not (Table XVI) include the doubtful determinations, species of probable recent introduction, &c.

26. The proportion of Monocotyledons is greatest in the Aucklands, least in the Kermadecs, and intermediate in the Chathams.

27. Stewart Island shows a greater proportion of Monocotyledons than New Zealand.

28. Species reaching both Aucklands and Chathams reach also in general to Auckland city.

The evidence is against the probability of carriage by water of South American forms to New Zealand, or of New Zealand forms to South America, though there are a few that may have been so carried, and favours the former existence of a land bridge, which probably passed to New Zealand nearer to the Aucklands than to the other southern islands.

29. The families missing in the Chathams are the smaller families of the Stewart list, in general; (30) similarly the smaller genera.

31. A great part of the Chathams' flora can be predicted by aid of age and area.

32. The Chathams have more species in the highest classes (widest distribution in New Zealand) than the Kermadecs and Aucklands.

The hypothesis has now been successfully used to make no fewer than 67 predictions, which have proved to be correct on examination of the facts, and increase of area with age may thus perhaps be considered as being the chief positive factor in determining the distribution of plants about the globe, the chief negative factor being the presence of barriers.

Finally, a restatement of the hypothesis is made.

LITERATURE QUOTED.

1. BOWER: Natural Classification of Plants (Hooker Lecture), esp. p. 119. Journ. Linn. Soc. (Bot.), xliv, 1918, p. 107.
2. CHEESEMAN: Manual of the Flora of New Zealand, Wellington, 1906.
3. ——————: Islands to the S. of New Zealand, in Chilton's Subantarctic Islands of New Zealand, ii. 389, Wellington, 1909.
4. COCKAYNE: Botanical Survey of Stewart Island, Wellington, 1909.
5. GUPPY: Plants, Seeds, and Currents in the West Indies and Azores, 1917.
6. WILLIS: Distribution of Species in New Zealand. Ann. Bot., xxx, p. 437, 1916.
7. ——————: Relative Age of Endemic Species. l. c., xxxi, p. 189.
8. ——————: Distribution of Plants of the Outlying Islands. l. c., xxxi, p. 327.
9. ——————: Further Evidence for Age and Area. l. c., xxxi, p. 335.
10. ——————: Sources and Distribution of the New Zealand Flora. l. c., xxxii, p. 339.
11. ——————: The Flora of Stewart Island. l. c., xxxiii, p. 23.
12. ——————: Studies in the Morphology of the Podostemaceae. Ann. R. B. Gardens, Peradeniya, i, p. 267, 1902.
13. ——————: The Endemic Flora of Ceylon: A Correction. Proc. Roy. Soc., B., lxxxix, p. 257.

Studies on the Chloroplasts of Desmids. II.

BY

N. CARTER, M.Sc.

With Plates XIX and XX and one Figure in the Text.

CONTENTS.

IX. THE CHLOROPLASTS OF *Micrasterias*.

IN this genus the general form of the chloroplast in some species has long been recognized. Thus an extensive chloroplast plate containing numbers of scattered pyrenoids was figured by Ralfs (1848) in several species, and later Delponte (1873) and W. and G. S. West (1904–11) also indicated the presence of ridges on the surface of the chloroplast in some other species.

But although it has long been observed that in those species of the genus having very flattened cells the chloroplasts are correspondingly flattened and plate-like, in the case of those species in which the cells are comparatively thicker very little is known concerning the chloroplasts.

The species whose chloroplasts have been examined during this investigation are *M. conferta*, Lund., *M. denticulata*, Bréb., *M. Thomasiana*, Arch., *M. rotata*, (Grev.) Ralfs, *M. papillifera*, Bréb., *M. radiata*, Hass., *M. Crux-Melitensis*, (Ehrenb.) Hass., *M. pinnatifida*, (Kütz.) Ralfs, *M. apiculata*, (Ehrenb.) Menegh., *M. Americana*, (Ehrenb.) Ralfs, *M. oscitans*, Ralfs, var. *mucronata*, (Dixon) Wille, and *M. truncata*, (Corda) Bréb. In spite of the fact that the chloroplasts of these species present very diverse appearances when examined in the front view, it has been found that they are all built up on the same plan. On comparing, for instance, the chloroplasts of a species having very flattened cells, e.g. *M. denticulata*, with those of a species whose cells are in proportion very much thicker, e.g. *M. truncata*, considerable differences are at once apparent; cf. Pl. XIX and XX, Figs. 2 and 25; nevertheless both can be considered as variations of one simple type.

There is in every case one chloroplast in each semi-cell, and this consists primarily of an extensive flattened plate, having approximately the same outline as the cell-wall, and occupying a central position, its surfaces parallel to the front faces of the semi-cell. As a rule from both sides of this axile plate various ridges are given off, stretching towards the cell-wall. The size, number, and disposition of these ridges vary considerably according to the species, and even to some extent amongst individuals of the same

[Annals of Botany, Vol. XXXIII No. CXXXI. July, 1919.]

species, according to the physiological condition of the cell. This is particularly the case with some of the flat-celled species, where frequently in some individuals no trace of ridges is to be found at all, whereas in others they are quite distinct. In other thicker celled species, e.g. *M. truncata*, they are often so large, numerous, and profusely branched, that the axile plate is frequently quite hidden by them. The size and prominence of the ridges depends largely on the thickness of the cell, because they stretch out towards the cell-wall, chiefly at right angles to the axile plate.

In the flat-celled species of *Micrasterias* the axile plate does not usually occupy more than one-third the entire space between the two front walls of the semi-cell (Figs. 3–8 and 10), but the thickness of the chloroplast always varies considerably according to the amount of stroma starch contained in it. The axile plate extends into all the peripheral lobes and projections of the cell-wall, and is slightly hollowed out at the base of the semi-cell for the accommodation of the nucleus (Figs. 2, 9, and 24). In outline it may be very similar to the cell-wall, or it may be even more intricately incised and lobed. Sometimes it seems to be drawn out at intervals into threadlike strands which apparently connect up the chloroplast to the peripheral layer of cytoplasm lining the cell-wall. This happens when the chloroplast is not sufficiently massive to extend right up to the cell-wall at all points.

In those species with very thick cells the axile plate is not so evident in the front view as in the case of those species having flattened cells (Figs. 13, 19, and 25). It is seen, however, in the end view or in transverse sections as an extremely thin plate lying in the middle of the cell, halfway between the front walls (Fig. 27). Towards the sides of the cell it usually loses its identity in the profusion of branching ridges (Figs. 14, 20, 21, and 27). Very often in such cases it has no definite plate-like form, but consists of a more or less irregularly shaped mass connecting up the huge ridges one with another, these forming by far the more important part of the chloroplast (Fig. 21).

In nearly all the species examined a more or less extensive hollowing away of the central axile plate was noticed in the upper region of the polar lobe. This is often seen as a semicircular or more elongated space free from chloroplast in the extreme apex of the cell. In the flat-celled species this apical hollowing rarely extends for more than one-fourth the distance from apex to nucleus, and is frequently less (Figs. 2 and 24). In the thick-celled species, however, the axile plate is sometimes shortened from the apex by as much as two-thirds its greatest length, so that the chloroplast is distinctly bilobed (Figs. 15, 19, and 25). When this happens the ridges often spread round over the internal surface of the wall so that the absence of the axis in the apical region may not at first sight be noticed, but in

other cases the colourless gap may be quite obvious. In such individuals there are often apparently two chloroplasts in each semi-cell, separated from each other by a colourless space in the median region. On careful examination, however, it is usually possible to locate the remains, at any rate, of the axile plate just beyond the nucleus connecting up the two apparently separate chloroplasts (Fig. 25). In the genus *Micrasterias* the formation of two separate chloroplasts in a single semi-cell by means of this extensive hollowing away in the apical region was not very frequently observed, but in *Cosmarium Ralfsii*,[1] which has a chloroplast exactly similar to that of the thick-celled species of *Micrasterias*, the same phenomenon also occurs, and here all stages in the progressive shortening of the axis in the median region have been observed, including the final stage in which two distinct chloroplasts were present. It is quite possible that this also happens occasionally in those species of *Micrasterias* having very thick cells.

The ridges, if present, project from both surfaces of the axile plate, extending to the cell-wall. If the distance from the central axis to the cell-wall be considerable, i.e. in the thick-celled species, the ridges frequently fork and branch (Figs. 20–22 and 27); in most cases, however, they are simple and unbranched (Figs. 14 and 18). Their free edges are rarely quite straight, but either undulate (Figs. 2, 15, and 17), or, as is more often the case, they are much more elaborately ornamented (Figs. 13 and 24). Sometimes their edges may be drawn out to form short thread-like outgrowths attached to the peripheral layer of cytoplasm lining the cell-wall, as in the case of the edges of the axile plate (Fig. 19).

In many of the flat-celled species the ridges project only very slightly from the surface of the plate, and in some individuals may be scarcely perceptible. When this is the case they are usually to be found following the outlines of the more important incisions of the plate, just a little distance from its edge (Figs. 2 and 24).

Very often there are two very prominent ridges on both surfaces of the axile plate in the median region. These usually extend right from the nucleus at the base of the semi-cell up into the polar lobe, one on each side of the apical gap in the axis (Text-fig.; Figs. 9, 15, and 17). They usually stretch for a little way over the surface of the nucleus itself, which is therefore embraced by eight claw-like projections, the outgrowths of the four ridges on each chloroplast of the cell. In species having such prominent median ridges other smaller ones may or may not be present. If present they are sometimes irregularly scattered (Figs. 13 and 15), or may be in definite positions round the more important incisions as before (Text-fig.).

[1] This species was for some time believed to have chloroplasts in the form of parietal bands; its axile form was first discovered by Dr. Lütkemüller, who figured it and published a short note on it in his paper, Zur Kenntnis der Desmidiaceen Böhmens, 1910.

In those species of the genus having the thickest cells the ridges are comparatively speaking very large and profusely branched. Their arrangement is usually very irregular, and they extend in all directions towards the cell-wall (Figs. 19 and 25). Here the axial part of the chloroplast is quite insignificant, and it is not surprising that in the apical and median regions of the cell the axis is often wanting.

It is probable that this absence of chlorophyll from the apical region of so many individuals is a manifestation of the attempt on the part of the organism to fill the more peripheral parts of the semi-cell with photosynthetic material as soon as possible, at the risk of robbing the interior of its normal share, since the parts of the chloroplast underlying the cell-wall are obviously more important in the process of photosynthesis than the more internal parts. This theory is supported by observations of the behaviour of the chloroplasts during cell-division. When the young semi-cell is newly formed it contains at first no chlorophyll, but soon the chloroplast of the older semi-cell begins to bud, sending two lobes through the isthmus, one on each side of the nucleus into the young semi-cell. In the very flattened cells of some species this bilobed form is not retained in a very conspicuous manner as the chloroplast streams more and more into the new semi-cell, and very soon the median part between them also begins to bud, so that the ingrowing chloroplast becomes practically semi-circular in outline, excepting for a small gap often found in its extreme apex. This is because all parts of the cell are equally good for photosynthesis, and it is just as important that the median part of the cell should be provided with chlorophyll as the more lateral parts. But in the thicker celled species the two small lobes first projected into the new semi-cell quickly expend their substance in an attempt to mantle the cell-wall by the formation of numerous large ridges which spread out in all directions as more and more chloroplast enters from the older semi-cell. Whilst this is happening the axis has very little chance of developing in the median part of the cell, as indeed there is little need for it to do so, since the median part of the front walls is probably already sufficiently mantled by ridges extending from the left and right lobes of the chloroplast. Thus it often happens that when the division of the chloroplast occurs at the isthmus, the chloroplast in the new semi-cell is still distinctly bilobed, or, if the growth of the two chloroplast lobes has been so rapid that the axis in the median part has not been able to develop at all, then these two lobes become completely isolated when separated from the original chloroplast by the division at the isthmus, and consequently the new semi-cell contains two distinct chloroplasts instead of one.

The pyrenoids in the chloroplasts of this genus are often extremely numerous (Figs. 2, 9, and 17), but at the same time they are very variable both in size and number. In individuals of the same species, for instance,

there may be thirty fairly large pyrenoids, or as many as eighty to a hundred rather smaller ones.

It is in those species having compressed cells and a simple axile chloroplast plate that the pyrenoids are most numerous, and here they are scattered indiscriminately. In the thick-celled species, where the ridges of the chloroplast have become relatively more important, the pyrenoids are reduced in number, and are more or less confined to those parts of the axis which give rise to the ridges (Fig. 27), whilst in those species having branched ridges they may be found also in the more peripheral parts of the ridges at the points of branching (Figs. 21 and 22).

M. conferta.

This species has a rather simpler chloroplast than any other species examined. Only one specimen was available for examination, and this had in each semi-cell a simple axile plate of very irregular shape. Ridges were only very slightly developed, and were found only along a few, not all, of the larger incisions. The pyrenoids were eight or nine in number; they were variable in size and scattered irregularly (Fig. 1).

M. denticulata and *M. Thomasiana.*

The chloroplasts of these two species greatly resemble each other. There is a large axile plate which is usually provided with a series of more or less distinct, though comparatively small, ridges following the outlines of the more important incisions between the lobes (Figs. 2–8). The prominence of the ridges, however, varies considerably with different individuals. This variation is again to be correlated with the amount of stroma starch present in the individual. If little starch be present, then the ridges along the incisions of the cell will usually be very distinct, whereas, if the chloroplast becomes very distended with stroma starch, the axile plate is often swollen to three or four times its former thickness and the ridges are lost.

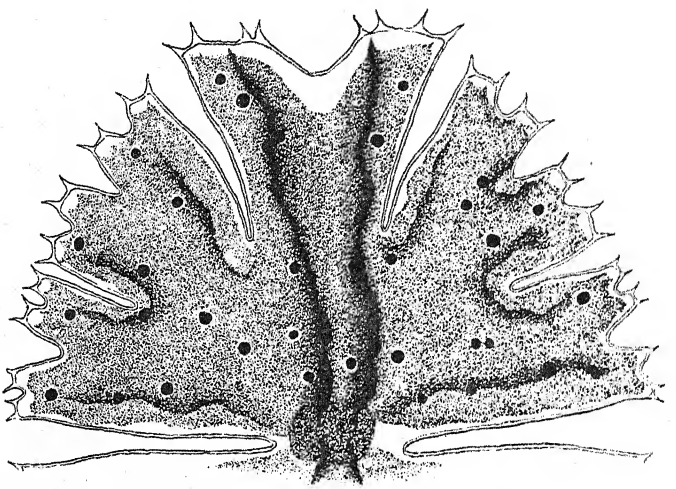
In *M. Thomasiana* the ridges parallel to the sinus project for some distance into the two outgrowths of the cell-wall at the base of the semi-cell; the third or median lobule was not strongly developed in the material examined.

In both species the pyrenoids are very numerous, there being usually about sixteen to seventy in *M. Thomasiana*, and twenty to forty in the case of *M. denticulata* (Fig. 2). Occasionally in the latter species there may be more than a hundred pyrenoids in a single chloroplast.

M. papillifera.

In this species there is a series of very distinct ridges arranged all round the incisions of the axile plate (Fig. 24). They are fairly large, very

sharply defined, and have their free edges produced to form outgrowths of various shapes. The two median ridges continue for some distance beyond the incisions on each side of the polar lobe, but never extend quite as far as the nucleus. The pyrenoids are large and number from twelve to fifteen.



M. apiculata, (Ehrenb.) Menegh. × 510.

M. rotata and *M. apiculata.*

The most striking feature of the chloroplasts of these two species is that there are two very prominent ridges on each side of the axis stretching right from the nucleus to the apex of the chloroplast. These are rather larger in *M. apiculata* than in *M. rotata* because of the relatively greater thickness of the cell; cf. Text-fig. with Figs. 9 and 10. In the former species there is in addition a series of ridges, almost equally striking, following the outlines of the principal incisions of the axile plate. In *M. rotata* ridges are usually wanting in the lateral lobes of the cell, and even if present they are only very slightly developed. The pyrenoids in *M. apiculata* usually number about thirty; in *M. rotata* they are often much more numerous, and may be more than eighty.

M. radiata and *M. Crux-Melitensis.*

The chloroplasts of these two species are quite similar to each other (Figs. 15, 17, and 23). The axile plate extends for quite a considerable distance into the peripheral outgrowths of the cell-wall, and has two rather definite median ridges projecting from both its surfaces. There are various other ridges (Fig. 18), very irregularly arranged, perhaps more so in the case of *M. radiata* than in *M. Crux-Melitensis.* In the only specimen of the latter species examined there were about thirteen pyrenoids in each semi-cell, whilst in *M. radiata* they vary from eight to thirty.

M. pinnatifida.

In this tiny species two very prominent median ridges are to be seen in the front view, and towards the lateral edges of the cell the plate thickens abruptly, at the same time splitting into two lamellae. This is best seen in the end view (Fig. 12). It was only possible to examine one individual, and this had three pyrenoids in each semi-cell, one in the polar lobe and one in each lateral lobe of the cell (Fig. 11).

M. Americana.

This species has ridges which are very large and conspicuous, but probably unbranched. There are two very distinct median ridges visible in the front view, but these usually seem to be displaced either to one side or the other (Fig. 13). This is due to the fact that the front faces of the cell are not flat, and the cell is much thicker in the middle than at the edges (Fig. 14). Consequently the cell always tilts over to one side when resting on a flat surface. The peg-like outgrowths of the cell-wall on the polar lobe are usually the cause of this tilting to one side of the cell, but in the variety examined these were not strongly developed.

Besides the median ridges of the chloroplast there are various others radiating from the region of the nucleus towards the periphery. In spite of the thickness of the cell, the central axis, as in all the other species dealt with so far, retains its definite plate-like form. In Fig. 14 the axis is seen to be very distended with stroma starch, which has not yet, however, spread as far as the ridges. The pyrenoids number about twelve to fifteen and are scattered throughout the axis of the chloroplast.

M. oscitans, var. *mucronata*, and *M. truncata.*

These two species have relatively thicker cells than any of the others which were examined. In both the ridges are variable in number, but they are always large and very much branched, quite superseding the central axis in their much greater development (Figs. 21 and 27). The delicacy of the branching of the chloroplast is largely dependent on the amount of stroma starch present in it. The formation of much starch causes the chloroplast to become very distended, and thus the elegance of the stroma starch-free chloroplast is lost; cf. Figs. 20 and 21.

The shortening of the axile plate in the median region of the cell is frequently considerable, and the chloroplast is in consequence often distinctly bilobed (Figs. 19 and 25). In such cases a transverse section near the top of the semi-cell is quite different from one near the nucleus. The former naturally cuts through the two separate lobes of the chloroplast, and two distinct masses are to be seen (Fig. 22), whilst towards the base of the semi-cell a continuous central axis is seen with various plates or branched

ridges stretching towards the cell-wall in all directions (Fig. 21). In the front view two median ridges are usually quite conspicuous in comparison with the others, and that part of the central axis lying between them, even where it is present, is often very delicate and only found after careful focusing (Fig. 25). Occasionally specimens of *M. truncata* have been observed in which two distinct chloroplasts were present in each semi-cell, the axis in the median region of the cell being altogether absent, but as a rule the remains of the axile plate can be seen just above the nucleus.

When there is a very extensive hollowing away of the axis in the upper part of the semi-cell, the ridges in the apical region often spread themselves closely over the inner surface of the cell-wall, forming a more or less definite parietal layer (Figs. 25 and 26).

The pyrenoids in both species vary very much in size, and usually number about five to ten in each semi-cell, although there may be as many as twenty. They are usually to be found embedded in the thicker parts of the chloroplast, where the ridges arise from the central axis, or even in the more peripheral parts of the ridges themselves. Occasionally pyrenoids occur in the parietal part of the chloroplast in the apical lobe where the axis in this region is wanting (Fig. 26), but the majority of the pyrenoids are always to be found in the interior of the cell. They are very often seen to be in a state of active division, two or more small ones being constricted off from a single large one.

Summary of the Special Characters of *Micrasterias*.

In all the species examined there is normally one chloroplast in each semi-cell, and all the different forms encountered can be considered as variations of one simple type.

There is always a more or less distinct axile plate parallel to the front faces of the semi-cell in the central position, and from this there usually arise numbers of ridges or plates which project towards the cell-wall in various directions. The relative size of the axis and ridges varies with different species.

The prominence of the ridges seems to depend on the thickness of the cell; in the more flattened cells of some species they are insignificant and may under certain physiological conditions be altogether absent, but in the thicker celled species they are very large and may sometimes even be branched. The ridges in the latter case constitute by far the more important part of the chloroplast, the axis often becoming very thin and indefinite in form.

With the increase in size and importance of the ridges, the pyrenoids become more restricted in number and disposition, occurring only in the more massive parts of the chloroplast.

In most species a tendency was noticed for the axis of the chloroplast to become shortened in the apical lobe of the cell. This is more pronounced in the thicker celled species, in which the chloroplast may even tend to become parietal in this region by reason of the absence of the axis. Sometimes the shortening of the axis in the median region extends through the whole length of the semi-cell, in which case two chloroplasts are present instead of one.

In conclusion, I wish to express my gratitude to the Birmingham Natural History and Philosophical Society and also to the Royal Society for grants to help in the cost of reproducing the plates illustrating this work. My thanks are also due to Professor G. S. West for much valuable help and advice throughout the investigation.

THE BOTANICAL LABORATORY,
UNIVERSITY OF BIRMINGHAM.

BIBLIOGRAPHY.

DELPONTE, J. B. (1873): Specimen *Desmidiacearum subalpinum*, Augustae Taurinorum. Memor. d. R. Accad. d. Scienze di Torino, Ser. 2, vol. xxviii, 1873.

LÜTKEMÜLLER, J. (1910): Zur Kenntnis der Desmidiaceen Böhmens. Verhandl. der k. k. zool.-bot. Gesell., lx, 1910.

RALFS, J. (1848): The British Desmidiaceae. London, 1848.

WEST, W. and G. S. (1904–11): Monograph of the British Desmidiaceae. Ray Society, vol. i, 1904; vol. ii, 1905; vol. iii, 1908; vol. iv, 1911.

DESCRIPTION OF PLATES XIX AND XX.

Illustrating Miss N. Carter's paper on *Chloroplasts of Desmids. II.*

During the prolonged processes of preparation the specific characters of Desmids often become obliterated, but all the species were identified, either in the living or carefully fixed condition, by Professor G. S. West.

PLATE XIX. (All × 510).

Fig. 1. *Micrasterias conferta*, Lund.

Figs. 2–8. *M. denticulata*, Bréb. Fig. 2, front view; Fig. 3, longitudinal median section; Figs. 4–8, transverse sections taken at intervals from the sinus to the apex of the semi-cell, showing the large axile plate, and the comparatively small ridges cut in various directions.

Figs. 9 and 10. *M. rotata*, (Grev.) Ralfs. Fig. 9, front view of semi-cell; Fig. 10, slightly oblique transverse section, showing the median ridges cut transversely.

Figs. 11 and 12. *M. pinnatifida*, (Kütz.) Ralfs. Fig. 11, front view; Fig. 12, optical transverse section.

PLATE XX. (All × 510).

Figs. 13 and 14. *M. Americana*, (Ehrenb.) Ralfs, var. *Lewisiana*, West. Fig. 13, front view; Fig. 14, optical transverse section, showing the axis of the chloroplast greatly distended with starch.

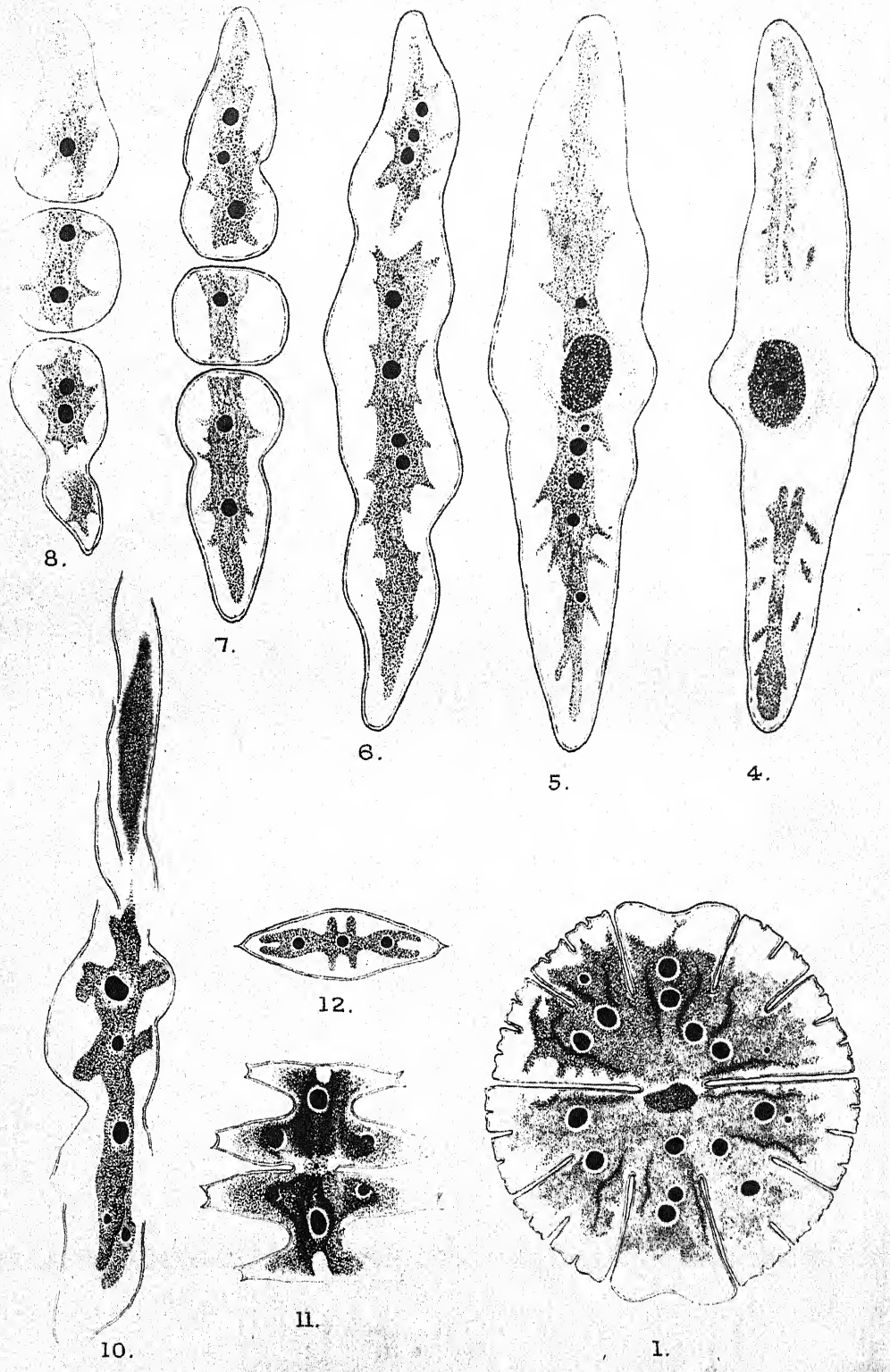
Figs. 15–18. *M. radiata*, Hass. Fig. 15, front view of semi-cell showing the bilobed form of the chloroplast owing to the shortening of the axis in the median region; Fig. 17, front view of another individual which does not show this phenomenon; Fig. 16, longitudinal oblique section, the ridges cut irregularly in surface section; Fig. 18, transverse section.

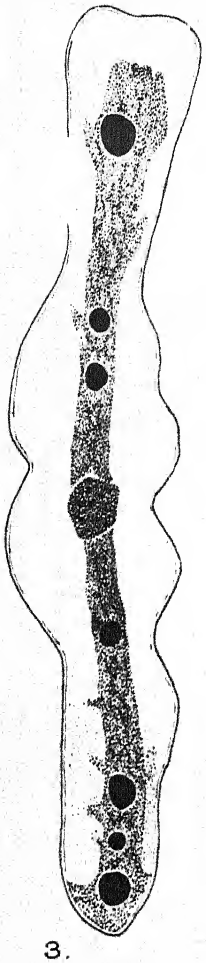
Figs. 19–22. *M. oscitans*, Ralfs, var. *mucronata*, (Dixon) Wille. Fig. 19, front view of individual having a distinctly bilobed chloroplast in each semi-cell; Fig. 20, transverse section showing the whole chloroplast distended with stroma starch; Fig. 21, transverse section of an individual whose chloroplast contains little starch excepting in the immediate neighbourhood of the pyrenoids; Fig. 22, transverse section of a specimen whose chloroplast is deeply cleft owing to the shortening of the axis in the median region.

Fig. 23. *M. Crux-Melitensis*, (Ehrenb.) Hass., front view.

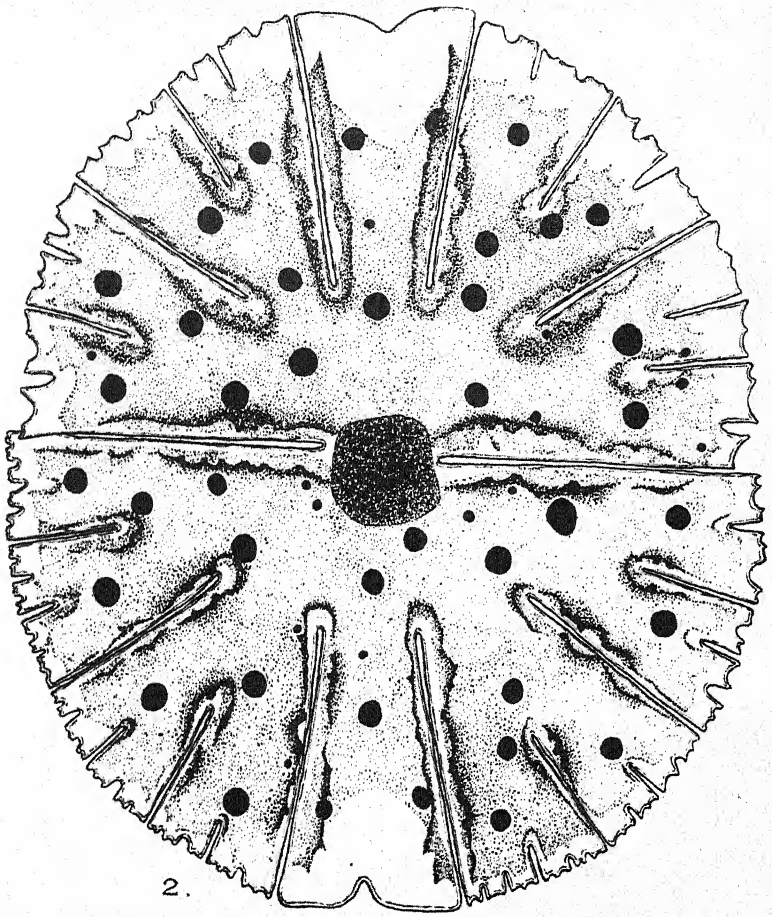
Fig. 24. *M. papillifera*, Bréb., front view.

Figs. 25–27. *M. truncata*, (Corda) Bréb. Fig. 25, front view of an individual, the axis of whose chloroplast is much shortened and very delicate in the median region; Fig. 26, surface view of the polar lobe of the same individual seen from the end, showing how the cell-wall is mantled with parietal films of chloroplast when the axis is wanting in this region of the cell; Fig. 27, optical transverse section of the basal part of the semi-cell.

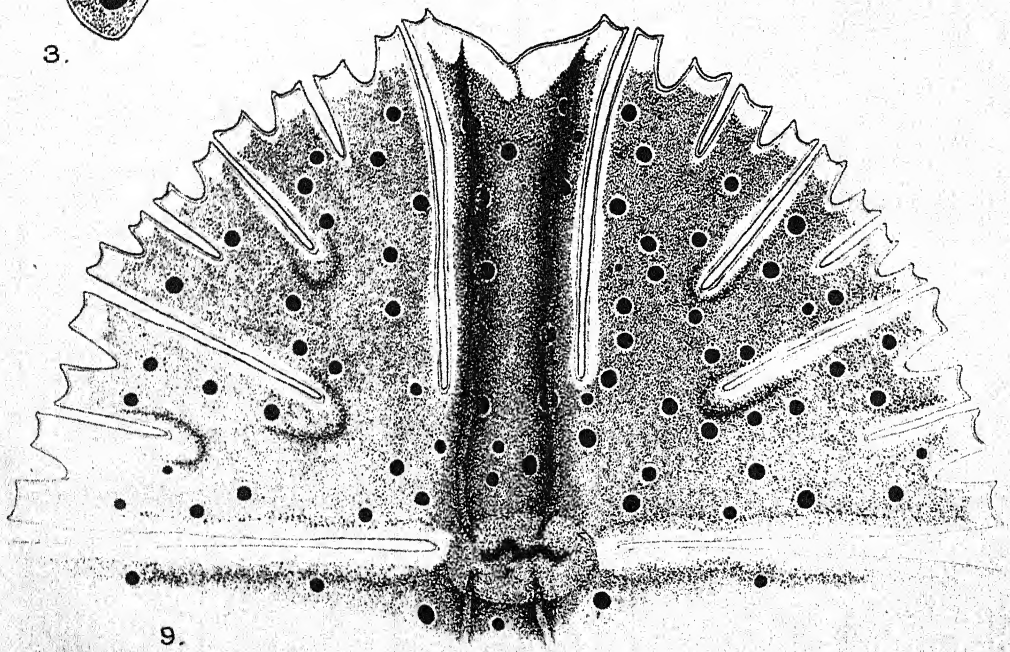

8.
7.
6.
5.
4.
12.
11.
10.
1.



3.

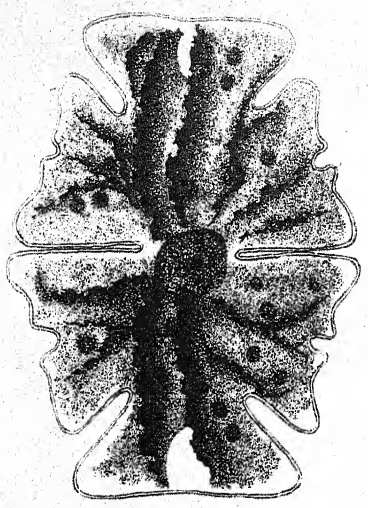


2.

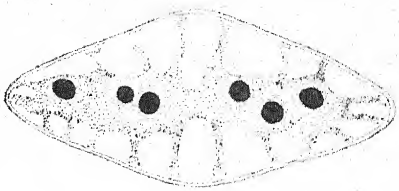


9.

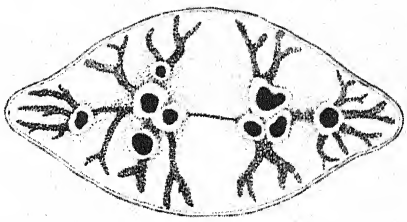
Huth, London.



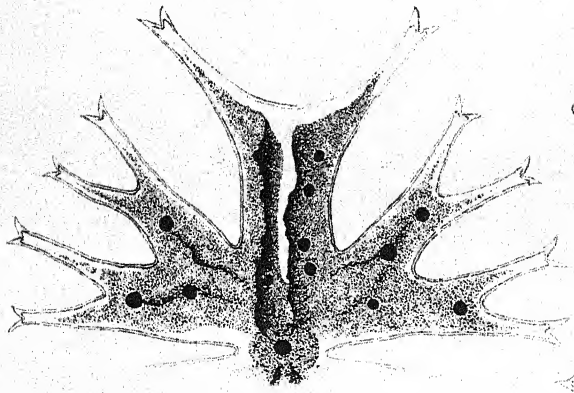
13.



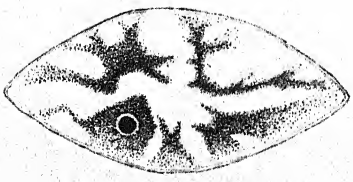
14.



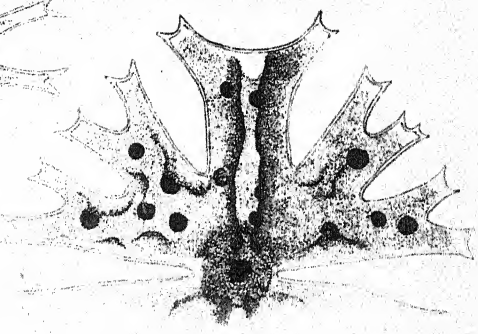
27.



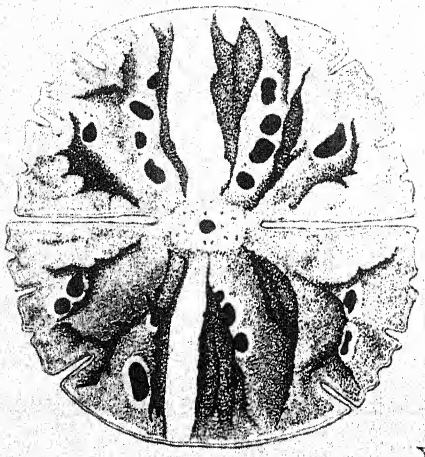
15.



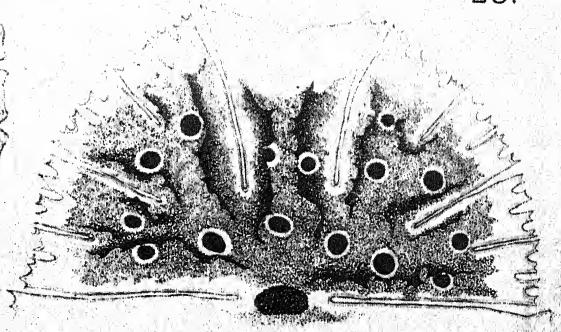
26.



23.

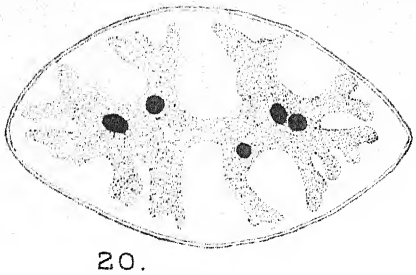


25.

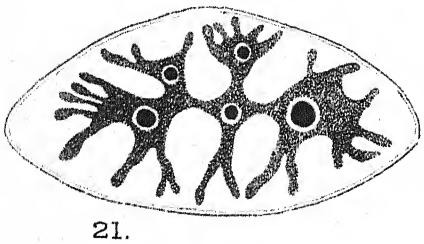


24.

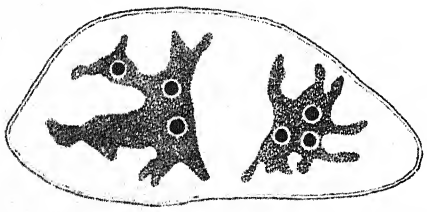
N. CARTER— MICRASTERIAS.



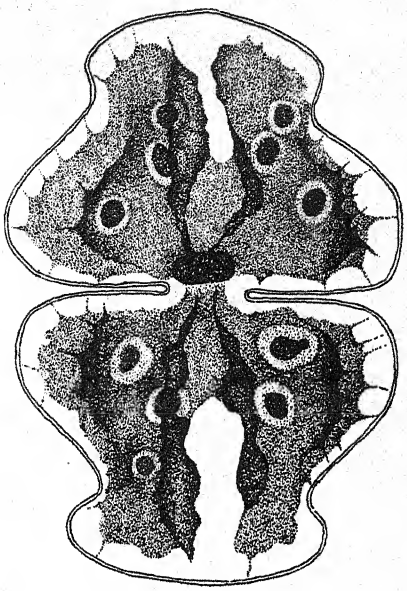
20.



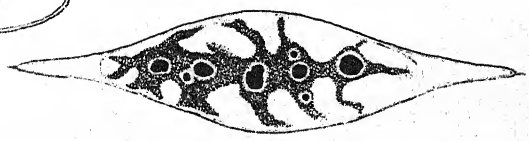
21.



22.

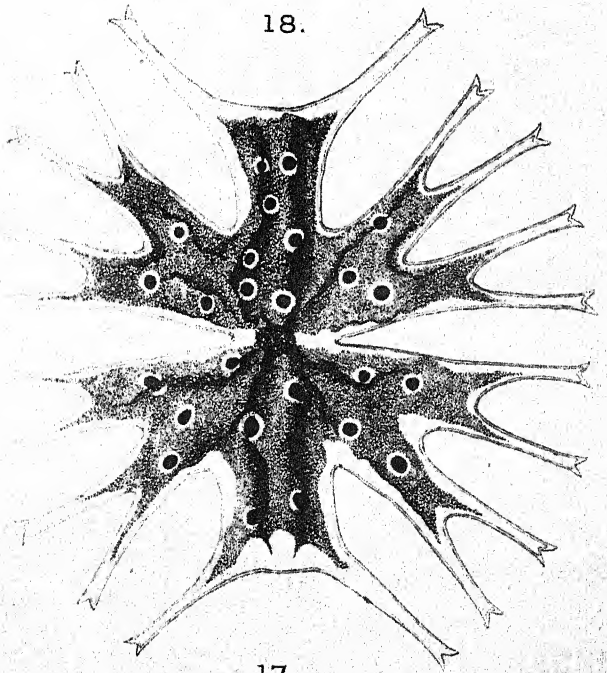


19.



18.

16.



17.

Huth, London.

Studies in the Physiology of Parasitism.

V. Infection by Colletotrichum Lindemuthianum.

BY

P. K. DEY, B.Sc.

From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.

With Plate XXI.

IN all previous works on bean anthracnose caused by the well-known parasite *Colletotrichum Lindemuthianum*, no observations of the actual mode of infection have been made. Whetzel (14) gives a diagram of penetration of the germ-tube through the outer surface of the bean-pod but he does not appear to have made any study of the details of penetration. Frank (6) observed that the germ-tubes of *C. Lindemuthianum* produced on the surface of the host dark-brown appressoria from which infection took place, but he neither described nor gave any figures of the process of infection.

In the case of infection by *Botrytis cinerea*, workers like Büsgen (4) and Marshall Ward (12), believed that the germ-tube effected its entrance by softening and dissolving the cuticularized epidermal wall of the host; Voges (11) also speaks of the slime formed by the germ-tube of *Fusicladium* softening the cuticle. But, as pointed out by Blackman and Welsford (1), no critical observations were in any case made. It was Brown (2) who, working with *B. cinerea*, first showed that the cuticle of the host is quite unaffected by the powerful extract which he obtained from young hyphae. When such an extract is placed on a delicate, uninjured rose petal, the cuticle and the underlying tissue remain unchanged; when placed on wounded petals, however, disorganization of the tissue takes place in a very short time. He thus indicated that the germ-tubes of *B. cinerea* have no power of causing a softening of the cuticle, and that the latter is impermeable to the enzymes which dissolve the non-cuticularized walls. It thus seemed probable that penetration of the cuticle by the germ-tube takes place by mechanical means. Blackman and Welsford (1) made careful microscopic study of this question, and showed that the passage of the germ-

[Annals of Botany, Vol. XXXIII. No. CXXXI. July, 1919.]

tube of *B. cinerea* into the bean-leaf was effected by a rupture of the cuticle, due to the mechanical pressure exerted by the germ-tube.

The object of the present work was to make a careful microscopic examination of the stages of infection of the bean by *Colletotrichum Lindemuthianum*, and to discover if this fungus, belonging to a quite different group, acts in the same way as *Botrytis cinerea*.

Methods. The cultures were made mostly in a medium of maize-meal agar, also on French bean-pods, autoclaved in test-tubes. The maize-meal agar medium was made in the following way—30 grm. of maize-meal were cooked in 1,000 c.c. water at 100° C. for half an hour. To this 15 grm. of agar were added, and the mixture heated to 110° C., to dissolve the agar. The medium thus prepared was 'tubed' and autoclaved at 120° C. for 20 minutes.

Spores were sown on slopes and Petri dishes. In three to four days small white patches appear. Examination of the young growth shows a felted mass of fine hyphae, with indications here and there of formation of acervuli. The hyphae frequently swell up into vesicles, as described by Stoneman (9), and branch copiously, giving a knotted appearance to the mycelium. After six days these spreading patches were dotted over with very small black areas, which even with a hand lens were seen to consist of very dark spines enclosing a pinkish spore mass. The spores are borne on short erect hyphae, which arise from swollen vesicles, and are abjointed from the tips of the hyphae, two or three in a series. At the same time mucilage is formed which surrounds the spores. After a few more days of growth acervuli become still more numerous, and gradually turn the white background of mycelium into a black mass. The spore masses are now visible to the naked eye as round pinkish globules. The hyphae of the fungus are colourless, but the dark appearance of an old colony is due to the black stiff spines of the numerous acervuli. In cultures a month old or more, the spore masses, instead of remaining as moist pink globules, seem to dry up and shrink, becoming at the same time rather creamy in colour. They also become set with dark-coloured spines, so that old cultures become almost uniformly black and rugged in appearance.

Germination of Spores. Spores from cultures about two weeks old were sown in thin films of sterile tap-water on a clean and flamed slide kept in a Petri dish containing sterile, moist blotting-paper. Under these conditions spores germinate quite readily in 18–24 hours at 20°–25° C.—the optimum temperature for the growth of the fungus, according to Edgerton (5), lying somewhere between 21°–23° C. It was observed that if the drop of water containing the spores had a sharp convex surface, spores lying in the middle of the drop hardly germinated, while most of those lying near the border did; this difference of behaviour is probably due to difference in oxygen supply. During the early stages of germina-

tion the spores swell up and become rather constricted in the middle, and thus have a more or less dumb-bell shaped appearance, as observed by former workers; they sometimes become septate. A germ-tube comes out generally from one end of the spore, but the rare, septate ones produce a germ-tube at each end. In a very few cases more than two germ-tubes were found to develop from a spore. The germ-tube, as it grows, comes in contact with the hard surface of the glass, and as soon as this occurs its tip swells out into a dark-brown, thick-walled, spore-like body, the appressorium (of Frank) or adhesion organ. Muncie (10) holds that the appressorium is not formed until the germ-tube has reached a certain length. Observation shows, however, that it is formed whenever the germ-tube touches a hard foreign substance; thus it may be produced soon after the germ-tube comes out of the spore, so that it lies almost in contact with the spore (Pl. XXI, Fig. 3 A); on the other hand, the germ-tube may grow to a considerable length before it is produced (Fig. 3). As to the stimulus necessary for its formation, there is no doubt it is the result of contact, as Hasselbring (7) has shown in the case of *Gloeosporium fructigenum*. In this species Hasselbring observed a distinct germ-pore in the appressorium, through which a hypha later grows out. In *C. Lindemuthianum*, however, no such germ-pore is to be found, the hypha always arising at the point of contact of the appressorium with the glass, whatever orientation the appressorium may possess. The young appressorium is sheathed in a mucilaginous coat (Figs. 4, 4 A). This can clearly be demonstrated by staining with very dilute watery gentian violet for 30 seconds, or by mounting the germinated spores in 'collargol' (1). By means of this sheath the appressorium becomes attached to the surface, so that even a fairly strong jet of water does not remove it. In fixed and stained preparations this mucilage appears to be reduced by the dehydrating agents to irregular granules and threads, analogous to the similar structures observed in the germ-tube of *Botrytis cinerea* by Blackman and Welsford (1).

Penetration of the Germ-tube into the Host. In following the details of penetration by the young 'infection hypha', freshly picked, young, juicy pods of French bean (*Phaseolus vulgaris*, vars. Canadian Wonder and Brown Dutch) were used. These two varieties were found to be very susceptible to the disease. The size of the bean-pods varied from 5 to 8 cm. long by about ½ cm. wide. In such young pods the cuticle is thin, and so they were likely to prove suitable for infection studies. Young leaves were first tried, but as their cuticle and the outer walls of their epidermal cells are exceedingly thin, such leaves were found unsuitable for observation of the changes occurring in the cuticle and subcuticular layers during penetration. Spores were taken from a culture on maize-meal agar which had been at 25° C. for about a month; these were stirred thoroughly in 1 c.c. of sterile tap-water till the mucilage holding them together in

masses was removed and a uniform suspension was obtained. Ordinary tap-water was used instead of a nutrient solution, as used by Blackman and Welsford in similar experiments with *B. cinerea*, as infection readily occurs in this, the normal medium, under natural conditions. The density of the suspension was estimated with the naked eye, till a faintly milky appearance was arrived at.

Small drops of this suspension were placed on bean-pods, previously washed thoroughly with sterile water to remove dust and foreign spores, and kept in a moist chamber. Care was taken that the drops spread out in thin films, so that nearly all the spores might be under similar condition of oxygen supply. The pods were then incubated at 25° C. On the second day, that is twenty-four hours after sowing, small bits of the pod under the 'infection drops' (3) were cut out every four hours, and fixed, generally in Carnoy's fluid (absolute alcohol, 6 parts; chloroform, 3 parts; glacial acetic acid, 1 part), but sometimes in Flemming's stronger solution of half strength. The tissue underneath the 'infection drop' does not show any sign of infection during early stages, so that it is not possible to gauge by the eye the stage of infection reached. Now and then small brown spots were visible with the microscope below the 'infection drop'; Frank noticed those spots about twenty-four hours after sowing the spores, and held that they were due to infection. These spots, however, appear even when tap-water is substituted for the 'infection drops'. Thus they are not the result of infection, but may be due to osmotic disturbances.

The fixed material was embedded in paraffin, and in every case sections 4 μ in thickness were cut. Heidenhain's iron-alum haematoxylin and erythrosin were first used for staining, but as erythrosin does not bring out the cuticle, a counterstain, Sudan III, was employed instead. In making up this stain 0·01 grm. of Sudan III (Grübler's) is first made into a paste with absolute alcohol, and then made up to 5 c.c. with 95 % alcohol. It is allowed to stand for a few days, and then 5 c.c. of pure glycerine are added to it. Sections thus stained were mounted in glycerine jelly (10).

Observations. The germ-tube produced by the spore soon forms a brown, thick-walled, more or less spherical appressorium on the surface of the pod (6). The appressorium is pressed closely against the surface, the part in actual contact with the pod becoming somewhat flattened. The appressorium appears to be held on the surface by means of its mucilaginous sheath. The spore is also fixed in some way to the surface, though no mucilaginous envelope has been observed around it; further growth of the germ-tube thus causes the tube to curve up, since it is fixed at both ends (Fig. 5). Some pressure is thus exerted on the surface, and, no doubt as a result of this, a slight indentation of the wall of the epidermal cell is to be observed. This is the first preliminary to penetration.

In material on which spores have been growing for 24–40 hours, nothing beyond this preliminary stage is to be observed. The activities of the young germ-tube during this period are confined to the production of the appressorium, for the development of the infection hypha is never observed earlier than forty-eight hours after sowing, while the appressoria may be formed within twenty-four hours. Muncie held that the incubation period (between sowing and penetration) varied from $3\frac{1}{2}$ to 6 days, according to the amount of moisture present and the temperature. Edgerton was not able to obtain definite signs of infection earlier than four and a half days after sowing (8). These workers, of course, did not observe the very earliest stages of infection investigated here.

The next stage in infection is the development of a slight protuberance on that part of the appressorium in contact with the cuticular surface. This protuberance pushes in the cuticle still farther, so that a distinct hollow is formed on the surface (Fig. 5 A). From this protuberance there now develops a very fine peg-like outgrowth, the 'infection hypha', which stains homogeneously, and in which the distinction of wall and cavity could not be observed.[1] As the result of the development of the 'infection hypha' a rupture is caused in the relatively inelastic cuticle (Fig. 6). A very careful and searching study was made for evidence of disorganization or swelling or any other change in the cuticle at this stage or later, but no such evidence was obtainable. *C. Lindemuthianum* forms then no special substance which is capable of producing softening or chemical change in the cuticle. These facts prove that, as in the case of *B. cinerea*, the 'infection hypha' obtains entrance by breaking or rupturing the cuticle of the host as the result of the pressure exerted by the development of the 'infection hypha' arising from the appressorium. In sections this rupture is indicated by a break in the continuity of the cuticle near the peg (Figs. 7, 8, 10, 14, 15). The perforation to be observed is considerably wider than the 'infection hypha' itself. This may be explained by the fact that the epidermis of the bean-pod is no doubt in a state of tension, owing to the turgescence of the inner tissue.[2] When the 'infection hypha' enters through the cuticle and brings about softening and disorganization of the subcuticular layers, the cuticle is free to contract at that particular spot, and consequently the aperture is widened.

Once the cuticle is ruptured, the pressure due to growth in length of the germ-tube forces the appressorium a little farther into the surface of the pod; this is well seen in Fig. 15. In the same figure it is to be noted that the penetrating peg, which is at first exceedingly fine, swells into a hypha of normal size soon after it reaches the softer cellulose layers.

[1] The relation of the 'infection hypha' to the inner and outer layers of the appressorium could not be determined.

[2] That the outer tissues of the fruit are so stretched can be well demonstrated in the cucumber.

The passage of the fungus through the cell-wall is associated with a swelling and dissolution of the cellulose layers. The first sign of this action on the wall is the disappearance of the uniform stratification of the cell-wall, and the appearance of a clearer area round the 'infection hypha'. No such change in the subcuticular layers is to be noticed, however, before the break in the cuticle is effected. The cuticle apparently bars completely the passage into these layers of any enzyme secreted by the 'infection hypha'. The behaviour of *C. Lindemuthianum* is in this respect quite analogous with that of *B. cinerea*.

The 'infection hypha', after growing a short distance into the host, produces at its end a small vesicle (Figs. 9, 10, 11, 12, 13, 14) from which one or more branches emerge and spread through the host tissue. This vesicle may be developed in the swollen subcuticular layers of the wall, or its formation may be delayed till the 'infection hypha' has penetrated far into the cell. This vesicle appears to be similar to that described by Marshall Ward (13) for the infection process in *Uredo dispersa*; the nature of this vesicle is obscure.

The effect of the entrance of the hypha into the host-cell is to be observed in Figs. 14, 15, 16. From these figures it is evident that the collapse of the cell immediately beneath the place of entry takes place only after a hypha is well established in its cavity; the invading hypha may be the original 'infection hypha' (Fig. 15) or a branch from the 'vesicle' (Fig. 16). When the 'infection hypha' enters the cavity of the cell, the protoplasmic contents of the latter apparently flow towards the hypha and collect round it (Figs. 13, 14, 15). Movement of nuclei, similar to that found by Blackman and Welsford in the bean-cell invaded by *B. cinerea*, has never been observed in this case.

The stomata of the host do not seem to afford an easier channel of infection than that through the epidermal cell. Only one instance of the passage of an 'infection hypha' through the stoma was found in all the material examined. Even there infection has taken place from an appressorium in the usual manner, and not directly by the germ-tube (Fig. 17).

In conclusion, the author wishes to express his gratitude to Professor V. H. Blackman, at whose instigation the work was undertaken, and from whom he has throughout received most helpful criticism and advice.

Summary.

The spore of *Colletotrichum Lindemuthianum*, when germinating on the host plant, produces a germ-tube, which directly it comes in contact with the host surface develops at its end a thick-walled, dark-coloured appressorium. The appressorium becomes attached closely to the surface by the help of its mucilaginous envelope.

From the surface of the appressorium in contact with the host, a peg-like 'infection hypha' grows out, which ruptures mechanically the cuticular layer, and then brings about swelling of the subcuticular layers, no doubt by enzymic action.

There is no evidence that the 'infection hypha' can exert any swelling, disorganizing, or other chemical action upon the cuticle.

The mechanism by which the 'infection hypha' of *C. Lindemuthianum* penetrates the host surface is thus in all respects similar to the mechanism employed by the germ-tube of *Botrytis cinerea*.

References.

1. Blackman, V. H., and Welsford, E. J.: Studies in the Physiology of Parasitism. II. Infection by *Botrytis cinerea*. Ann. Bot., vol. xxx, p. 389, 1916.
2. Brown, W.: Studies in the Physiology of Parasitism. I. The Action of *Botrytis cinerea*. Ann. Bot., vol. xxix, p. 313, 1915.
3. ————: Studies in the Physiology of Parasitism. III. On the Relation between the 'Infection Drop' and the Underlying Host Tissue. Ann. Bot., vol. xxx, p. 439, 1916.
4. Büsgen, M.: Ueber einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit., vol. i, p. 53, 1893.
5. Edgerton, C. W.: Effect of Temperature on *Glomerella*. Phytopath., vol. v, p. 247, 1915.
6. Frank, B.: Ueber einige neue, weniger bekannte Pflanzenkrankheiten. Land. Jahrb., vol. xii, p. 511, 1883.
7. Hasselbring, H.: The Appressoria of the Anthracnoses. Bot. Gaz., vol. xlii, p. 135, 1906.
8. Muncie, J. H.: Experiments on the Control of Bean Blight. Michigan Agri. Coll. Expt. Sta. Technical Bull., 38, 1917.
9. Stoneman, B.: A Comparative Study of the Development of some Anthracnoses. Bot. Gaz., vol. xxvi, p. 69, 1898.
10. Strasburger und Koernicke: Botanisches Praktikum, 5th ed., p. 809, 1913.
11. Voges, E.: Die Bekämpfung des *Fusicladium*. Zeit. f. Pflanzenkrank., vol. xx, p. 385, 1910.
12. Ward, H. M.: A Lily Disease. Ann. Bot., vol. ii, p. 319, 1888.
13. ————: On the Histology of *Uredo dispersa*, Erikss., and the 'Mycoplasm' Hypothesis. Phil. Trans., B., vol. cxcvi, p. 26.
14. Whetzel, H. H.: Some Diseases of Beans. Cornell Univ. Agri. Expt. Sta. Bull., 239, 1906.
15. ————: Bean Anthracnose. Ibid., 255, 1908.

EXPLANATION OF FIGURES IN PLATE XXI.

Illustrating Mr. Dey's paper on Physiology of Parasitism.

All figures except 1–4 A were drawn with the camera lucida, under Koristka $\frac{1}{15}$ in semi-apochromatic, oil-immersion objective, and No. 8 eyepiece. Figs. 1–4 A were drawn under Leitz $\frac{1}{12}$ in. oil-immersion objective, and No. 4 eyepiece.

The host tissue figured is that of the pod of *Phaseolus vulgaris*.

Fig. 1. Germinating spore. × 1050.

Figs. 2–3 A. Germinating spore, showing the appressorium: drawn from material fixed in picro-nigrosin after twenty-four hours of growth. × 1050.

Figs. 4, 4 A. Germinating spore, showing mucilaginous sheath round the appressorium: drawn from fresh material stained with dilute gentian violet. × 1050.

Fig. 5. Germinating spore attached to the epidermis. The germ-tube is curved and is fixed at both ends. × 1500.

Fig. 5 A. A slight protuberance on the appressorium causing an indentation of the wall of the host. The mucilage round the appressorium appears to be reduced to thread-like structures. × 1500.

Fig. 6. The 'infection hypha' has grown out from the appressorium and penetrated the cuticle. × 1500.

Figs. 7, 8. The 'infection hypha' is growing in the subcuticular layers after effecting its entrance by perforating the cuticle. The hole produced in the cuticle is somewhat wider than the 'infection hypha'. × 1500.

Fig. 9. A somewhat later stage than Fig. 8. Swelling and disorganization of the cell-wall is clearly seen. × 1500.

Figs. 10, 11. The 'infection hypha' is swollen into a vesicle in the disorganized cellulose layers. × 1500.

Fig. 12. A somewhat later stage than Fig. 11. The vesicle has given origin to a branch. × 1500.

Fig. 13. The vesicle and its outgrowth are visible. Swelling and disorganization of the subcuticular layers have taken place. The protoplasm of the epidermal cell appears to have accumulated in the upper part of the cell, probably as the result of the action of the invading hypha. × 1500.

Fig. 14. The 'infection hypha' has formed the vesicle after passing into the cavity of the cell. Disorganization of the wall and the protoplasm has occurred. × 1500.

Fig. 15. The 'infection hypha' has passed into the cavity of the epidermal cell. Disorganization of the protoplasmic contents is well advanced. The break in the cuticle is now much enlarged and the appressorium is pressed slightly into it. The 'infection hypha', which is very narrow at its base, has swollen into a hypha of normal size in the layers of the cell-wall below the cuticle. The protoplasm of the cell appears to have accumulated round the 'infection hypha'. × 1500.

Fig. 16. The 'infection hypha' passing into the cavity of a guard cell has swollen out into a vesicle, and has caused shrinkage and death of the cell. × 1500.

Fig. 17. The appressorium has formed over a stoma. The 'infection hypha' is growing down through the pore of the stoma. × 1500.

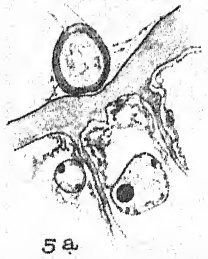
PUBLIC LIBRARY
29. AUG 1919

1.

2.

3.

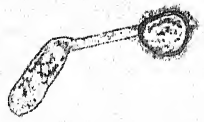
4.



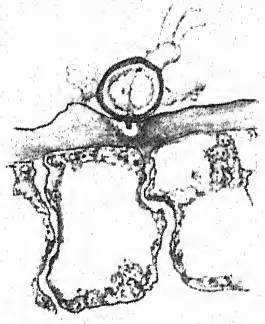
5a.

2a.

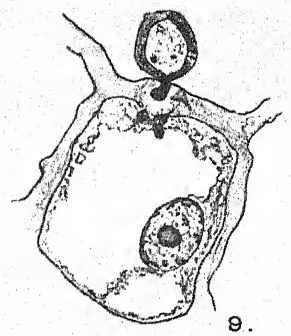
3a.



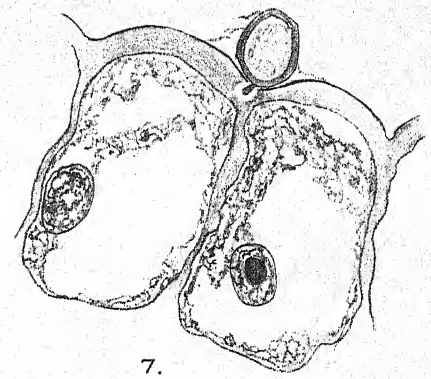
4a.



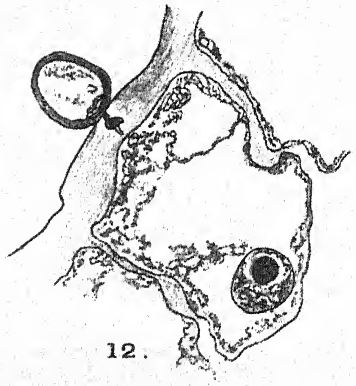
6.



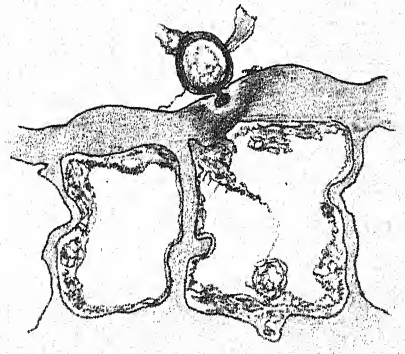
9.



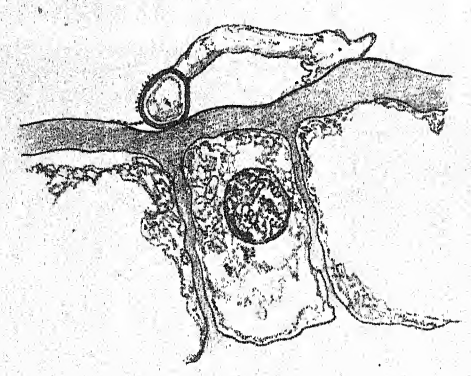
7.



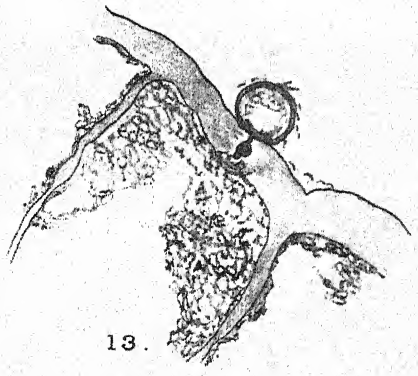
12.



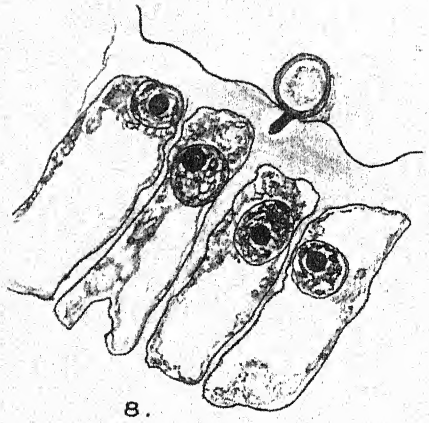
10.



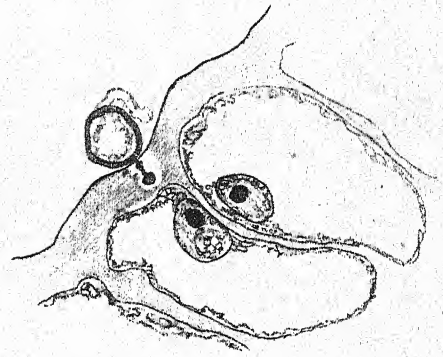
5.



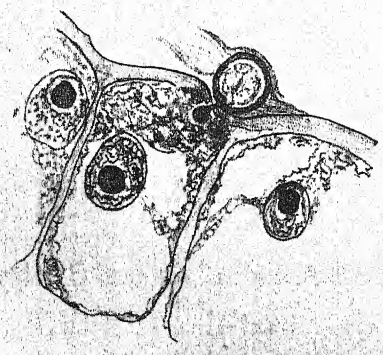
13.



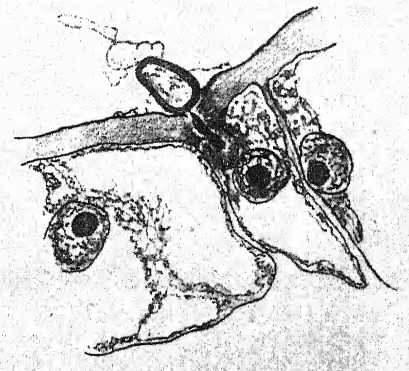
8.



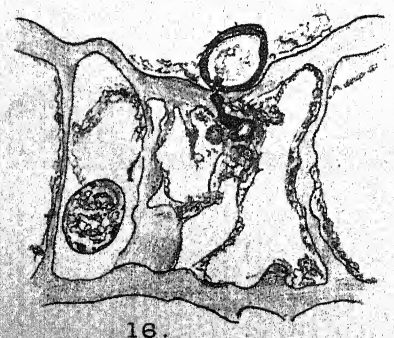
11.



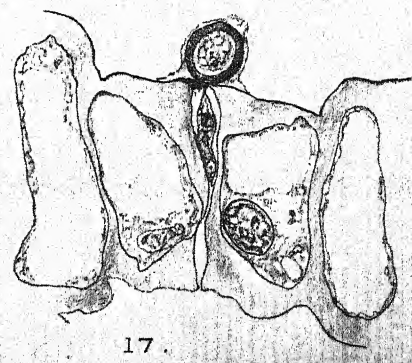
14.



15.



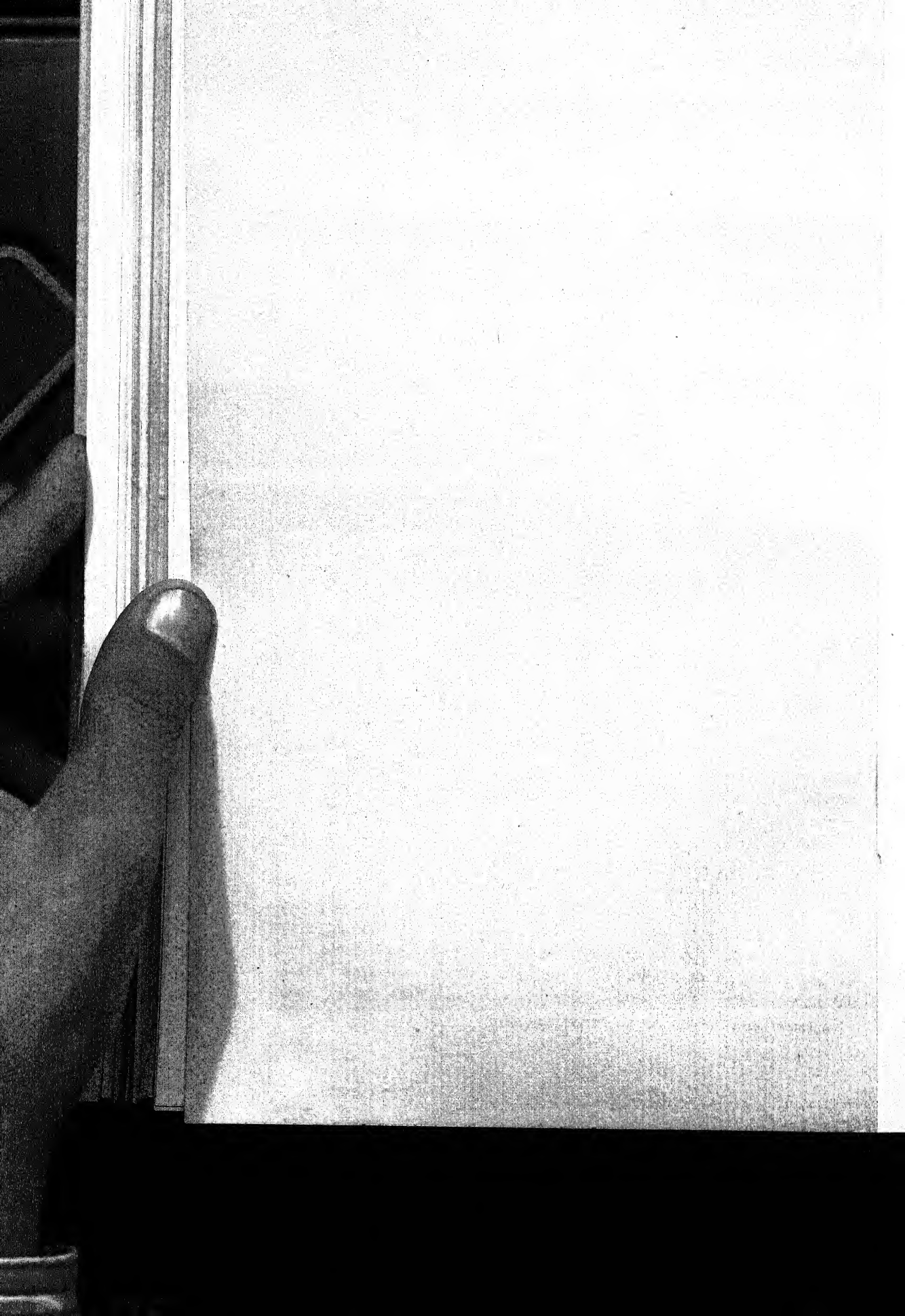
16.



17.

P. K. D. del.

Huth lith. et imp.



Variation in Hevea brasiliensis.

BY

STAFFORD WHITBY, M.Sc., A.R.C.S.,

McGill University, Canada.

With one Diagram in the Text.

IT was desired to secure data as to the extent to which variation occurs in the amount of rubber yielded by individual trees of *Hevea brasiliensis*, of the same age and growing under the same conditions, and as to the possible correlation between the yield of rubber and the girth of the trunk.

In addition to data directly relative to the matter just mentioned, the observations which have been made afford information concerning the extent of variation in the rubber content of the latex from individual trees and concerning a number of other points noticed in what follows.

The observations were made in the Federated Malay States. A normal area of plantation rubber trees, 7 years old, was selected. The area was approximately 13 acres in extent and contained 1,338 trees, planted 20 feet square. Of these trees 1,137 were in tapping. The trees comprising the balance of 201, not in tapping, were mostly 'supplies', i. e. trees which had been put in since the area was first planted up in order to fill vacancies caused by disease, pests, wind, &c. These trees were of course younger than the other trees on the area and, owing to overshadowing, had not had a good chance to grow. They were, in fact, all removed in the ordinary course of thinning-out operations on the plantation in question soon after the close of the observations recorded here. Of the 1,137 trees in tapping, 106 were one year behind the remainder in their tapping history; having attained a trunk-girth up to the standard fixed for the commencement of tapping operations about one year later than the rest of the trees. Some of them doubtless represented the original plantings, but others no doubt represented 'supplies'. As no means were available for distinguishing original plantings in this group from 'supplies', and as these trees were being tapped at a lower point on the trunk than the rest, they are not included in the present survey. Of the remainder of 1,031 trees, 20 are not included because of incompleteness of the record or failure of the tappers to cut deep enough. The data refer, therefore, to a population of 1,011 trees, in their third year of tapping, on a normal plantation area of seven-year-old Para rubber. They serve sufficiently well to indicate the extent of

[Annals of Botany, Vol. XXXIII. No. CXXXI. July, 1919.]

variation between individual trees, in regard particularly to rubber yield, on an area planted, as all the Eastern plantations have been, from non-selected seed, and to suggest the extent of possible improvement in the yields of plantations which seed selection might bring about.

Variation in the Rubber Content of Samples of Latex.

In order to decide as to whether the rubber content of the latex (the 'strength' of the latex) was sufficiently constant to make it feasible to lighten the burden of collecting yield data from individual trees by measuring volumes of latex instead of taking weights of rubber, the strength of the latex from a number of individual trees in the population was determined. These determinations revealed unexpectedly great variations in the strength of the latex from different trees. A frequency table for the samples of latex from the 245 trees examined is given in Table I. In each case the latex was diluted with an equal volume of water, and coagulated with acetic acid; the coagulum was converted into crêpe, and the crêpe, after being allowed to dry in the air, was weighed.

It was found that the strength of the latex from a given tree was approximately constant on different days,[1] and appeared to be characteristic for the individual tree. Several trees were kept under observation for more than a year; and it was found in these cases that, although, as was expected, the strength of the latex from a given tree changed to a certain extent with the weather, yet a tree yielding at one time latex with a rubber-content clearly higher than the average could be relied upon to yield strong latex at all times (and vice versa),[2] and that, by taking samples of latex from a number of trees over a short period such that weather conditions would not be likely to affect the trees to seriously unequal extents, a very fair comparison of the trees in respect of this characteristic could be made.

TABLE I.

Rubber Content of Latex from 245 Trees of Hevea brasiliensis, Seven Years Old.

Grm. rubber per 100 c.c.	23	24–25	26–27	28–29	30–31	32–33	34–35	36–37	38–39	40–41	42–43	44–45	46–47	48–49	50–51	52–53	54–55
Frequency	4	2	7	11	16	27	44	35	23	32	17	12	5	1	4	3	2

Mean = 36·58 ± 0·25 per cent.[3] σ = 5·86 ± 0·17 per cent. *C.V.* = 16·02 ± 0·49 per cent.

[1] The trees were tapped once a day only—in the early morning. It was found that when trees were tapped in the afternoon as well as in the morning the strength of the latex obtained on the second occasion was usually markedly lower than of that obtained on the first.

[2] Cf. Bulletin No. 13, Dept. Agric. Ceylon, 1914.

[3] This figure, it should be noted, refers to seven-year-old trees. The age of a tree is a factor in determining the strength of its latex. The author concluded from a limited number of observations, concerning areas from four to eighteen years old, that, as a tree grows older, the rubber content of the latex yielded by it increases 1–2 per cent. per annum.

The samples of latex included in the table were taken mostly from the larger yielding trees (mean yield 11·8 grm. per diem). The figures did not, however, indicate that there was any correlation between yield and latex strength. The figures may thus be considered as fairly indicative of the whole population in regard to the strength of the latex.

Variation in Yield.

The exact magnitude of the yield of rubber given by any one tree varied at different times, but was found to be, in general, sufficiently constant to allow of a fair comparison of the yielding capacities of the trees on a given area under similar tapping conditions being made by taking the mean of the yields on a number (in these observations, mostly six, but in some cases ten to twelve) of separate days.

It seemed clear that, although the actual magnitude of the yield from a given tree varied to a certain extent at different times, owing to variations in fortuitous circumstances, such as the rainfall, the prevailing humidity, the depth of tapping, the hour at which tapping is performed, yet trees presented characteristic differences in their capacity for yielding rubber, and, at all events in a first investigation, might reasonably be compared in respect of this capacity by taking measurements under conditions such that variations in the fortuitous factors which influence the yield were as far as possible avoided, or affected all trees equally, and by, in addition, basing conclusions for any given tree on the mean of several determinations.

In addition to the evidence as to the comparative constancy of the yield from individual trees, which the considerable body of data summarized below afforded when examined in detail, further evidence in support of this conclusion was secured by keeping a limited number of trees under observation for a longer period than that covered by the main series of measurements. In the case of some of these trees the yield was determined at intervals over a period of two years. It was clear that, although seasonal variations in the yield took place, the yield for a given tree was in most cases approximately constant over such a period. Speaking generally, a tree which was seen to be a high yielder at one time could be relied upon to give a high yield at all times.[1]

Concerning the factors which, apart from the characteristic yielding capacity, affect the yield, the following points are noted:

(*a*) All trees were under the same tapping system (a single V-cut on

[1] This conclusion may be considered as being in agreement with general plantation experience. Trees are sometimes noted as falling off seriously in their yield or even as 'going dry'. (It is possible that such behaviour may be due to disease or other abnormal conditions.) But, in general, it is recognized that high yielding trees (which naturally come under notice more frequently than other trees) can be expected, if not over-tapped, to give high yields during the whole of their tapping history.

Also cf. the records for the original generation of trees raised from the seeds first introduced into the East from Brazil: Bulletins Nos. 4 and 13, Dept. Agric. Ceylon.

half the circumference, reopened every morning) and had had the same tapping history. They had been tapped for two successive years on the two halves of the 'basal' section of the trunk, i.e. the portion extending vertically from, say, 20 in. to 4 in. from the ground. In the succeeding year of tapping, when the observations were made, they were tapped on the section of the trunk immediately above the 'basal' section, i.e. the section from, say, 36 in. to 20 in. from the ground. On all trees the tapping cut had advanced roughly the same distance down the section, viz. 6–8 in., at the beginning of the survey.

Thus the height of the tapping cut on the trunk did not enter, as a disturbing factor, into the observations.

(*b*) The trees were tapped by an experienced gang of tappers[1] by means of the simple gouge. The depth of the tapping on each tree was tested by a probe on each occasion that latex was collected from it. In the case of trees where the tapping was clearly not deep enough, the latex was not collected, and an instruction was issued to the tapper to go deeper in the future. The percentage which had thus to be dealt with was not very large. Finally, the records showed a small number of trees on which the tappers had not succeeded in going sufficiently deep, and, as already mentioned, these were excluded from the population surveyed.

It may be considered that the depth of tapping on the trees surveyed represented the greatest accuracy in this direction which is practically attainable.[2]

(*c*) In order to avoid as far as possible the disturbing effect on the comparison of variation in weather conditions, not only was the figure for the yield from each tree based on several measurements, but the times at which the measurements for each tree were taken were distributed at different points over the period of the observations. The period of the year at which the trees lose their leaves was avoided for the observations, as trees are very unequally affected by this 'wintering'. The observations were made over what may be regarded as four typical months in the

[1] It may fairly be mentioned that the native tappers show a degree of skill with the tapping gouge and an accuracy in excising thin strips of bark greater than any to which the author, personally, could lay claim.

[2] An attempt was made to classify the trees according to the degree of exactness with which the fullest possible depth of tapping consistent with avoidance of injury to the cambium had been attained, by estimating the extent to which further latex, if any, issued forth on probing at the tapping level; but it did not prove to offer any particular advantage in regard to the main object of the investigation. It appeared that, excluding the cases already mentioned in which the tapping was palpably not deep enough, the trees, on the one hand, which would, on the ground that deepening of the incision entirely failed to give more latex, be classed as being tapped fully deep enough were in general those giving small yields, and the trees, on the other hand, which would, on the ground that deepening of the incision gave somewhat more latex, be classed as not being tapped quite deep enough were in general those giving large yields. Thus, it may be remarked, a still more exact adjustment of the tapping depth than that actually attained would merely have had the general effect of increasing the contributions from the large yielders, and hence of making still more clearly marked the characteristic features of the variation noted later.

Malay States, viz. August–November. The element in the weather which most markedly influences the yield is the rainfall, the trees yielding better in wet than in dry weather. The daily rainfall on the area during the course of the observations was recorded. The monthly totals were: Aug., 4·73 in. (rain on 13 days); Sept., 12·83 in. (18 days); Oct., 6·72 in. (17 days); Nov. 9·46 in. (17 days).

The results obtained for the rubber yield from the trees surveyed are summarized in the following frequency table:

TABLE II.

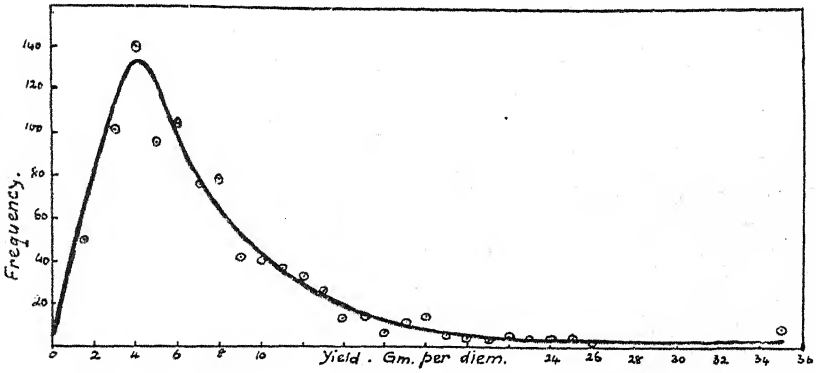
Rubber Yield from Population of 1,011 Seven-year-old Hevea brasiliensis.

Grm. per diem	0–1·5	2	3	4	5	6	7	8	9	10	11	12	13	14
Frequency	55	84	101	140	95	104	76	78	42	41	37	34	26	13

Grm. per diem	15	16	17	18	19	20	21	22	23	24	25	26	27 and over[1]
Frequency	15	7	11	14	5	4	3	4	4	3	4	2	9

Mean yield = 7·12 ± 0·115 grm.[2] σ = 5·425 ± 0·08. Mode (by inspection) = 4·0 grm. *CV.* = 76·19 ± 1·14 per cent. Coefficient of skewness (on σ) = + 0·575.

The results are represented graphically in the following diagram:



The outstanding feature of the variation in rubber yield among the population examined is sufficiently indicated by the high coefficient of variability, and the marked positive skewness of the frequency curve, viz. the presence of an important number of trees which are yielding amounts of rubber several times larger than the modal value.

[1] Mean of this group, 35·74 grm.

[2] Equivalent to 5·49 lb. per tree per annum. Making due allowance for the effect of wintering, for the time occupied by the establishment of wound response, &c., this figure accords fairly with the yield of 360 lb. per acre per annum which the section of the plantation in question was giving over all.

Lump rubber, i. e. natural coagulum which forms in the collecting cups, is included in the yields recorded, but 'tree scrap', i. e. rubber obtained from the latex which is left on the cuts after the trees have ceased to drip, is neglected. The average amount of tree scrap which each tapping gives is indicated by the following figures: (*a*) 195 trees gave 134 grm. tree scrap; (*b*) 190 trees gave 115 grm. tree scrap.

It may be remarked, for example, that 9·6 per cent. of the trees (trees giving twice the mean quantity or more) was on the average yielding 3·6 times as much rubber per tree as the remainder of the trees. On the one hand, 9·6 per cent. of the trees on the area was contributing 28 per cent. of the total yield. On the other hand, 13·7 per cent. of the trees (Groups 0–2 gms.) was contributing only 2·9 per cent. of the crop, and certainly did not repay the cost of tapping. The highest yielders in the population were four trees giving the following yields per diem: 41·45, 41·56, 41·72, 42·77 [1] grm.

The great possibilities of seed selection in improving rubber yields are indicated by the above figures.

The data obtained have a certain bearing on the conduct of tapping experiments. They show that the extent of variation on what is presumably a normal area may be such that it is quite impermissible to assume, as is often done in tapping experiments, that small groups of trees (say 50 or 100 trees) chosen at random from an area of uniform age, situation, and appearance, will have the same yielding capacity, and hence that differences in yield which the groups may display when different tapping systems are applied to them are due to differences in the tapping systems, and afford a true comparison of the yielding capabilities of the systems in question. Thus, taking a point at random on the area with which the present investigation deals, counting off from it 36 rows, and arranging these rows in their order of succession, into groups of 3, 6, and 12 rows, representing respectively groups of 45, 90, and 100 trees we find the yields per group, under the same tapping system, to be as follows: [2]

TABLE III.

Yields from Adjoining Groups of Trees, under the same Tapping System, on an apparently Uniform Area.

	Grm. rubber per diem.
Group of 45 trees.	284, 285, 204, 259, 260, 334, 392, 439, 328, 390, 325, 276.
„ „ 90 „	569, 463, 594, 831, 718, 601.
„ „ 180 „	1032, 1425, 1319.

[1] Equivalent to a yield (calculated without making allowance for the reduction which 'wintering', &c. involves) of 33 lb. for a tapping year of 350 days.

It may be remarked that at an earlier point in its history one of these four trees had, according to the estate records, been treated against an attack by white ants. It would seem certain, however, that the high yield which it displayed during the period of the present observations was not due to white ants, because (*a*) careful examination failed to reveal the presence of white ants and the tree was certainly alive two years after the observations had been concluded, (*b*) the greatly increased flow of latex which white ants are recognized as inducing is transient, whereas the present tree was found to give large yields over a period of two years during which it was kept under observation.

[2] The most frequent number of trees in a row was fifteen. In cases where the number was other than this, the yield for the row has, for the sake of simplicity, been adjusted proportionately.

A number of observations were made on the problem of collecting seeds from selected trees.

Owing to the circumstance that the seeds are often projected to considerable distances from a tree when the ripe capsules burst, the seeds lying on the ground under a given tree are not usually all derived from the tree in question. In order to secure seeds whose origin was definitely known, the author at first placed conical bags of wire-netting round the capsules on the selected trees; but it was later found sufficient to secure one true sample of the seeds from a given tree, as it was observed that the seeds from any one tree were exactly similar in appearance.[1] The differences which the seeds from different trees exhibit in regard to tint, mottling, and size,[2] are very marked, but perhaps even more noteworthy than such differences is the accuracy with which the tint, the mottle-pattern, and the shape, down to such peculiarities as slight striations on one side, are repeated in all the seeds from a given tree. With a little experience it was found possible, once a true sample had been secured to act as a pattern, readily to pick out the seeds from a given tree from those lying on the ground in its neighbourhood. It was often possible to avoid the trouble of placing a number of wire-netting bags on the tree for the purpose of securing a true sample by keeping the tree under watch for a short time on a hot afternoon at the period when the capsules were bursting in numbers.

Variation in Girth.

The girth of the trees was measured at two points: 22 in. and 36 in. from the ground. The girths recorded in the following frequency table are the mean of the girths at these two points.[3]

TABLE IV.

Girths of Population of 1,011 Seven-year-old Hevea brasiliensis.

Girth in cm.	50–52	53–56	57–60	61–64	65–68	69–72	73–76	77–80	81–84	85–88	89–92	93–96	97–100	101–104	105–108	109 and over.
Frequency	2	12	29	49	70	106	114	146	127	114	91	60	49	20	8	14[4]

Mean girth = 80·30 ± 0·25 cm. σ = 11·91 ± 0·18. *C.V.* = 14·85 ± 0·22 per cent.

[1] For a similar observation made in Brazil, cf. Cramer: Rubber Recueil, Amsterdam, 1914, 12.

[2] For data on the variation in weight and size, see Sprecher: Bull. Jardin Botanique de Buitenzorg, No. 19, 1915, p. 112.

[3] 22 in. from the ground represents the lower level and 36 in. from the ground the upper level of the section of the trunk which was being tapped during the year in which the observations were made. 22 in. from the ground is also the level frequently fixed for measuring the girth of trees in order to decide whether they are large enough for tapping; and it may therefore be stated that the mean girth given in the table was on the average 5·75 per cent. less than the girth measured at 22 in.

[4] Mean of this group, 113 cm.

The girth data, like the data for latex strengths, appear to present a normal distribution.

Correlation between Yield and Girth.

The question of the extent to which the girth of the trunk is indicative of the rubber yield of a tree has considerable practical significance. If the correlation results showed that it is justifiable to assume that there is a high degree of probability that a tree with a small trunk will give a poor yield, and a tree with a large trunk a good yield, the work of selecting trees, in regard to their yielding capacity, for thinning-out operations would be greatly facilitated.

The figures given below indicate that, although there is a definite positive correlation between yield and girth, the extent of the correlation is not sufficient to justify very much emphasis being placed on girth when selecting trees for thinning out.

TABLE V.

Correlation between Yield and Girth in a Population of 1011 Seven-year-old Hevea brasiliensis.

Yields, grm. per diem.

Girth, cm.	0–2	3–4	5–6	7–8	9–10	11–12	13–14	15–16	17–18	19–20	21–22	23–24	25–26	over 26	
50–52	1	1													2
53–56	5	3	2	1	1										12
57–60	8	11	3	5		1	1								29
61–64	14	15	8	6	3	2	1								49
65–68	13	24	16	6	3	4	2	1						1	70
69–72	16	30	25	17	7	5	2	2	1	1					106
73–76	17	28	19	17	11	14	2	2	1		2			1	114
77–80	17	36	32	21	14	11	8	4	1			1		1	146
81–84	16	27	29	23	8	6	5	1	6	2		1	2	1	127
85–88	13	28	21	16	8	9	5	2	5	3	1			3	114
89–92	8	17	18	19	9	6	3	1	6	1		2		1	91
93–96	5	9	10	12	8	4	1	3	1		3	1	2	1	60
97–100	3	8	9	4	5	6	5	3	3	1		1	1		49
101–104	2	2	5	2	2	3	1	1	1				1		20
105–108				3	3		1					1			8
109–124	1	2	2	2	1		2	2		1	1				14
	139	241	199	154	83	71	39	22	25	9	7	7	6	9	1011

$r = +0.260 \pm 0.020$.

In addition to the characters which have been mentioned as showing, for a given tree, approximate constancy, certain peculiarities were observed in the cases of particular trees, which were also constant over considerable periods of observations, and which may probably be regarded as characteristic of the trees displaying them. Such were the following :

(*a*) Rapid discoloration of the latex. Rapid oxidative discoloration of the latex may be associated merely with insufficiently deep tapping. Thus

it may not infrequently be observed that the latex left on the tapping cut, after a tree has ceased to drip, darkens rapidly at the upper end of the cut, where there is a strong tendency for the tapping to fall short of the proper depth. But, quite apart from cases of rapid discoloration which could be avoided by deeper tapping, it was observed that in some cases rapid discoloration appeared to be characteristic of the tree. One tree, which showed this feature very strikingly, was kept under notice for five years, and was always seen to produce rapidly-discolouring latex, which gave an exceptionally strong reaction for peroxidase.[1]

(*b*) Tendency to rapid coagulation. In the case of two trees in the population with which this communication more particularly deals, a tendency to exceptionally rapid natural coagulation was observed in the latex, and persisted over a considerable period.

(*c*) A marked cream-straw colour appeared to be characteristic of the latex from a small percentage of trees. (This colour is to be distinguished from the transient yellow colour which latex from a new cut often has before wound response has established itself.) The latex in question has a noticeably 'rich' appearance. It was not, however, found in general to have a higher rubber content than the average.

POSTSCRIPT.—Since the above was in print, A. A. L. Rutgers[2] has given some interesting data which are in full accord with the observations (made in 1913), recorded in the present paper, as to the practical constancy of the yield from individual trees and as to the character of the distribution of the total yield over a group of rubber trees of equal age. Data for three areas are recorded by Rutgers.[3] As a result of comparing the classification of the trees made at the outset with that made ten to twenty months later, he concludes that 'good trees remain good, poor trees remain poor'. From his data for a group of 1,467 trees (mean daily yield per tree, 9·1 g.) it may be calculated that, at one extreme, 9·0 per cent. of the trees (trees giving 17 g. or more) produced 23·9 per cent. of the total yield, and, at the other extreme, 27·3 per cent. of the trees (trees giving 0–5 g.) produced only 7·2 per cent. of the total yield.

[1] Cf. Whitby: Koll. Zeit., 1913, vol. xii, p. 147.

[2] Selectie en Uitdunning. Archief voor de Rubbercultuur, 1919, 3, 105–123.

[3] It may be remarked that in the case of two of these areas the volume of latex, not the weight of rubber, per tree was the quantity measured. Also, in the instructions with regard to the collection of yield data for the purpose of selecting trees for thinning-out operations, it is directed only that the volume of latex shall be determined. It would seem, however, from the results recorded in the present paper on the 'Variation in Rubber Content of Samples of Latex', that this simpler procedure does not give exact results, and that it is necessary to weigh the rubber.

The Cytology and Life-history of Nemalion multifidum, Ag.[1]

BY

RALPH E. CLELAND.

With Plates XXII—XXIV and three Figures in the Text.

INTRODUCTION.

THE cytological situation in those red algae which develop tetraspores has become in the past few years fairly clear. The seat of chromosome reduction has been accurately determined as being in the tetraspore mother-cell, and the life-histories of several of the representative forms have been worked out. The conclusions from cytological investigations have received striking support from experimental cultures (Lewis, 1912 *b*, 1914), which have shown that sexual plants come from tetraspores and tetrasporic plants from carpospores; these two phases alternating in the life-history. The situation, however, in those forms which do not produce tetraspores, has not been thoroughly ascertained. The results obtained by Wolfe (1904) on *Nemalion multifidum*, and Svedelius (1915) on *Scinaia furcellata*, are contradictory and the group as a whole needs thorough investigation.

In the hope that I might be able to throw some light upon this situation, work was begun on *Nemalion* during the summer of 1916. The investigation has been carried on at the University of Pennsylvania, and at the Marine Biological Laboratory, Wood's Hole, Mass., under the direction of Prof. Bradley M. Davis. I wish to take this opportunity of thanking Dr. Davis for material, and for the constant help and criticism that he has so willingly given. I am also indebted to Professors George T. Moore and Ivey F. Lewis for much sympathetic assistance extended to me at Wood's Hole. Since the work was undertaken, a short paper on *Nemalion* by Kylin (1916 *c*) has appeared. A number of his results I have been able to verify.

METHODS AND MATERIAL.

Some of the material used in this investigation was collected by Dr. Davis in the summer of 1908 off Gay Head, Mass., and fixed by him in weak Flemming. This proved to be very well fixed. The rest of the

[1] Contribution from the Botanical Laboratory of the University of Pennsylvania.

[Annals of Botany, Vol. XXXIII. No. CXXXI. July, 1919.]

material was collected by the writer during the summer of 1917, at Wood's Hole, Mass., and vicinity. Three fixing reagents were employed—weak Flemming, weak chromacetic, and Merkel's fluid. The two latter were tried primarily as aids in the study of chromatophore structure, and for nuclear studies proved to be decidedly inferior. Fixations were made at all hours of the day and night, both in the field and in the laboratory. Some of the material was fixed at ordinary temperatures, some at close to 0° C. The latter material showed no improvement over the former.

Stages in the germination of carpospores were obtained by placing fruiting plants in shallow vessels in sea-water where the spores were shed for a number of hours. By scraping the bottoms of such dishes, a wide range of stages was obtained. Spores were also collected on slides laid in the bottom of dishes under fruiting plants.

Most of the material was embedded in paraffin and sectioned at 3 μ. Some was mounted whole, either in glycerine jelly, or fixed to the slide with Meyer's albumin, then stained and mounted in balsam. Heidenhain's haematoxylin was the stain chiefly used. Experiment proved the following procedure to be the most satisfactory for sectioned material: 4 per cent. iron alum, 8 hours; running water, 5 minutes; Heidenhain's haematoxylin, $\frac{1}{2}$ per cent. water solution, 36 to 48 hours; running water, 5 minutes; 2 per cent. iron alum until sufficiently destained. With weaker treatment the chromatic structures failed to take the stain strongly enough to give proper differentiation.

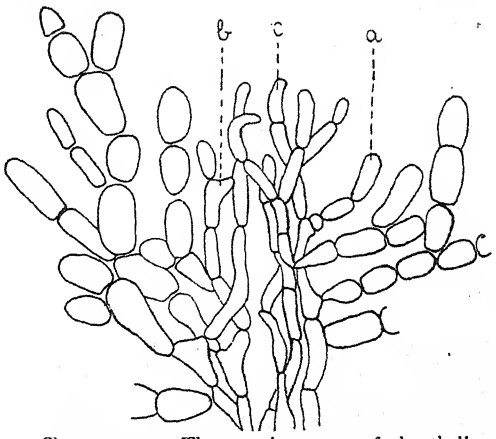
Vegetative Structure.

Although the vegetative structure of *Nemalion* is well known, a short account of certain details of anatomy and development should be given. The thallus consists of a central core of closely intertwining and branching threads from which radiate out in continuous series tufts of branching assimilative filaments.

The strands of the central core are largely made up of long, attenuate cells, in which I could find neither chromatophore nor nucleus, and which apparently serve to support and hold together the tufts of assimilative filaments. The tips of these strands, however, are actively growing and are found scattered throughout the region of the core, but especially in the younger parts of the plant. From these tips new assimilative filaments and new cells of the central core threads are developed in rapid succession (Text-fig. 1). The cells of these actively growing tips are short, thin-walled, and have a nucleus, but no chromatophore (Fig. 7). The strands lengthen by division of the apical cell and the branches which develop from them arise as lateral buds from cells near the apex (Text-fig. 1, *b*). As the cells become removed from the growing tips, the nuclei are less evident

and finally cannot be distinguished, the cells becoming typical mature strand cells of the central core.

At the tip of the thallus the central strands are very active, the terminal cells cutting off segments rapidly, from many of which new apical cells arise as lateral outgrowths. Some of the branches thus formed become new central strands, but most of them develop into assimilative branches (Text-fig. 1, *b*, *c*). So quickly does this development take place that the assimilative filaments completely surround and hide the central portion, and it is not unusual to find mature procarps within a distance of their own length of the tip. The nuclei of the apical strand cells are very large and present a strongly reticulate appearance. In the cells of filaments destined to continue as central strands no chromatophore can be found (Fig. 7), but these appear very quickly in the cells of developing assimilative filaments.

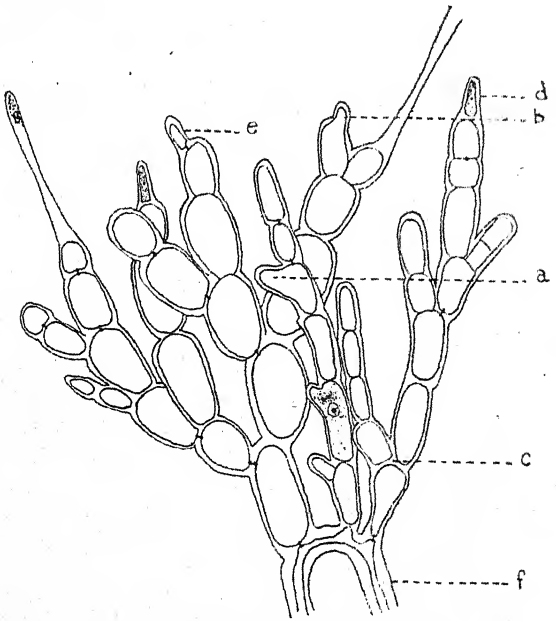


TEXT-FIG. 1. The growing apex of the thallus. *a*. Apical cell of a young assimilative branch. *b*. Lateral budding to form a new branch. *c*. Terminal cell of a central strand filament. × 700.

While most of the assimilative tufts arise at the tip of the thallus, new tufts are constantly being interpolated between old ones farther back. In the development of the tufts two methods of growth are present. First, growth may take place by apical cell division. This is the method by which all branches already formed increase in length. The apical cell lengthens until it is three or four times as long as broad. The nucleus usually divides before the chromatophore (Figs. 4–6), although in germinating carpospores the reverse is more often the case (Figs. 81, 90). Second, growth may be by budding. Buds may be of two kinds, those leading to the development of side-branches (Text-fig. 2, *a*), and those which give rise to trichomes (Text-fig. 2, *b*). The latter always grow out from a terminal cell, the former from a subterminal one. The bud which is to develop into a side-branch is put out as a papilla at the upper end of the cell. The chromatophore moves to the opening into the papilla and there divides, one-half passing into the bud. The nucleus divides, and one daughter nucleus passes into the new cell, which is then cut off. The new branch, thus begun, develops by apical growth, and, as it matures, gradually takes on an appearance of equality with the main branch, giving rise to a condition of false dichotomy. True dichotomy is not present in *Nemalion*.

Trichomes may continue the axis of the parent filament (Text-fig. 2, *d*), but more usually they are developed at one side of the median line of the terminal cell (Text-fig. 2, *e*). Each trichome is equipped with a nucleus, but has no chromatophore. After the trichome cell is cut off, it grows very rapidly and soon becomes fifty or more times as long as broad. At its distal end there is a quantity of cytoplasm in which lies the nucleus. The rest of the hair appears to be empty. After it has reached maturity, the base of the hair begins to gelatinize and a new trichome is developed to take its place.

It is important to notice, in relation to the development of the



TEXT-FIG. 2. Method of growth in a vegetative tuft. *a*. Lateral budding to form a side-branch. *b*. Terminal budding to form a trichome. *c*. False appearance of dichotomy. *d*. Trichome developed on the median line. *e*. Trichome developed to one side of the median line. *f*. Looped central strand filament. × 700.

chromatophore, to be described later, that the growth of the assimilative tuft is limited and that this limit is reached very close to the growing apex of the plant. Cells, therefore, which but a short way behind the apex are terminal cells of filaments, will contiuue to be terminal all through the history of the plant. The growth in thickness of the thallus, as it becomes older, is due to increase in the number of central strands; and the increase in assimilative structure which goes with this enlargement is due entirely to the development of new or secondary filaments, either from old tufts or from active strands of the central region.

Cell Structure.

The cell-wall is lamellate, as is easily demonstrated by the use of swelling reagents. The interior of a vegetative cell is very largely taken up by the chromatophore, which rests towards the upper end and occupies about one-half to two-thirds of the cell space. At the lower end, especially in old cells, there is usually a large vacuolar area. Below the chromatophore and between it and the vacuole lies the nucleus. It is very small, measuring usually about 2·5–3·5 μ in diameter. Within the nuclear membrane lies a conspicuous nucleolus. The remaining space within the nucleus is occupied by a faintly staining, but unmistakable reticulum (Pl. XXIII, Fig. 1). The nuclear cavity is never entirely empty of a reticular structure except in newly organized nuclei immediately following mitosis. At this stage, the small cavity is traversed only by a few radiating fibrillae which appear to suspend the nucleolus in the centre, the entire chromatin content being contained within this nucleolus. The details of mitosis in vegetative cells are very difficult to study, since the nuclei are small, and since, also, stages are not very often found, due to the fact that *Nemalion* is a slow-growing plant, only making eight to ten inches of growth in a season. The main details, however, have been made out satisfactorily, and it is certain that the chromosome number is about 8 as reported by Wolfe. Except for the absence of the nucleolus at metaphase, the details of nuclear behaviour (Figs. 1–3) are in entire correspondence with those seen in the cystocarp, where they are more easily studied. No detailed account, therefore, will be given at this time.

The cells are joined by protoplasmic connexions. These stain an intense black with Heidenhain's haematoxylin, so that they stand out very clearly. They appear as a pair of fused lens-shaped bodies, one to each cell, the edges of which are continuous with the inner surfaces of the cell-walls (Figs. 23, 40, 43). Owing to their small size and deeply staining nature, it has been impossible to demonstrate protoplasmic connexions as clearly as has been done by Lewis (1909) for *Griffithsia Bornetiana*. That intimate connexions exist, however, can hardly be doubted.

The Chromatophore.

The most striking object in the cell is the chromatophore. It consists typically (Figs. 9–12) of a central region containing a pyrenoid-like body, surrounded by a dense enveloping layer, from which strands radiate outward to the periphery of the cell, where they flatten out to some extent against the plasma membrane. These strands may be long, like spokes of a wagon wheel, or short, like cogs, according as the central region of the chromatophore occupies a larger or smaller portion of the cell. In either case, they flatten out in the same way at the periphery. Usually they do

not approximate one to another closely enough at the periphery to form a membrane.

It is the central region of the chromatophore, however, which is of greatest interest. The appearance of this region varies according to the position of the cell of which it is a part. If the cell lies at the periphery of the thallus, where it is in the best position for assimilation, the central region consists of a spherical or ellipsoidal dark-staining area (Fig. 9), either minutely granular or quite homogeneous and amorphous-looking, in the centre of which is an intensely staining pyrenoid body, of homogeneous appearance. In a cell which lies towards the interior of the thallus, however, we find quite a different condition (Fig. 11). Here the pyrenoid body is smaller and occasionally even altogether wanting. The homogeneous area surrounding it is replaced by a series of more or less granular threads radiating outward from the pyrenoid to the periphery of the central region. When the pyrenoid is entirely absent the threads occupy the entire central area. If we examine cells intermediate in position between the innermost and outermost (Fig. 10) we find all gradations between the two conditions just described.

We must not conclude, however, that the conditions shown in Fig. 9 will be gradually attained by the chromatophore in Fig. 11 as it grows older. It must be remembered that the growth of each assimilative filament is limited, and this growth limit is reached a very short distance behind the growing apex. The result of this is that the cell shown in Fig. 9, which is the terminal cell of a mature filament, will remain the terminal cell of that filament (except in so far as it may cut off one or more trichomes) throughout the life of the plant. Furthermore, the cell shown in Fig. 11, which is situated somewhat below the periphery of the thallus, although it was at one time the terminal cell of the filament, has, at all times, held its present position with reference to the interior of the thallus—that is, close to the central core. In other words, this cell has not had the same assimilatory advantages that the cell in Fig. 9 has had throughout its whole life. There is, therefore, no direct transition from the condition in Fig. 11 to that in Fig. 9. This is a natural result of the absence of intercalary growth.

The method of development of these two conditions in the chromatophore is illustrated in Fig. 8. Chromatophores make their appearance wherever one of the active strands of the core region gives rise to a new assimilative tuft. If we follow the development of a single tuft through from the apex, we observe that a cell is cut off laterally from a central strand. At first this cell shows no signs of chromatophore, but very soon a structure looking like a small area of very dense cytoplasm is seen (Fig. 8, *a*). This rapidly becomes denser and more distinct (Fig. 8, *b*). An aggregation of material then takes place towards the centre of the structure (Fig. 8, *c*), where it becomes arranged into threads, the peripheral part

meanwhile losing in density. Cell division occurs while the chromatophore is in condition *b* or *c*.

After the first cell division, the chromatophore left in the segment will follow the line of development shown in Fig. 8, *d*, *e*, since this cell is always to remain buried within the thallus. While the outer region of the chromatophore is growing in size and developing projections which reach to the periphery of the cell, the central region becomes larger and the threads become more open and loose. The pyrenoid then begins to appear, at first small, then increasing in size, until at maturity it occupies about one-half of the area.

The apical cell meanwhile continues to divide and re-divide until the limit of growth is reached. Division then ceases. The chromatophore of the apical cell is in stage *b* or *f* of Fig. 8. Stage *f* differs from stage *c* in that the central region is very densely granular and shows no tendency towards a thread-like arrangement. This chromatophore now takes the line of development *g*, *h*. As it enlarges, the outer zone puts forth projections in exactly the same manner as shown in *d*. The central region, however, becomes less granular and more homogeneous (Fig. 8, *g*). The pyrenoid then makes its appearance (Fig. 8, *h*) as a small, round, dark body, which rapidly increases in size until, in the mature cell, it has the appearance shown in Fig. 9. We see, therefore, that the chromatophore may develop in different ways depending upon its position in the thallus. If we were to trace the development of a chromatophore in one of the middle cells of the filament, we should find it taking a course midway between the two described. Every degree of difference can be found.

Division of the chromatophore is by simple elongation and constriction. If it is in the condition shown in Fig. 8, *c* or *f*, the granular portion constricts first, then the outer portion. Chromatophores which are older than this commonly do not divide, the only exception being where a mature cell gives rise to a side-bud which develops into a new filament. Everywhere else, however, including cystocarp and carpospore, the general rule holds.

The difference between the conditions which I have just described and the results which Wolfe obtained are striking. Wolfe found no pyrenoid structures of any kind. Instead, he reported the central region of the chromatophore as occupied by a vacuole, entirely empty of solid contents. He, therefore, concluded that *Nemalion* has no pyrenoid or pyrenoid-like structure. The difference between his results and mine are due entirely to methods of fixation. Wolfe used only chromacetic fluids as killing reagents, the effect of which seems to be to destroy more or less completely the material of the central region. I also employed weak chromacetic to some extent, as well as Merkel's fluid. With Merkel's fluid, an appearance almost identical with that shown in Wolfe's figures was obtained (Fig. 13), the central region nearly always being an empty hollow, the bounding wall

of which was covered with small granules. With weak chromacetic I obtained similar results. In no cell was the central region intact, and in many cases it seemed entirely empty (Fig. 14), as figured by Wolfe, although more usually a part or all of the pyrenoid or central body remained in an ill-preserved or shrunken condition (Fig. 15), with occasionally a few other granules scattered about in the surrounding vacuole. Perfectly preserved chromatophores were only found in Flemming-fixed material. It is clear, therefore, that a pyrenoid-like structure exists in the centre of the chromatophore. In this connexion, it is interesting to note Schmitz's (1882) statement with regard to the sensitivity of the pyrenoid of the Nemalionaceae and Bangiaceae: 'Bei diesen Algen nämlich quellen die Pyrenoide bei Einwirkung von süssem Wasser, Spiritus, verdünnete Essigsauer, u. s. w. auf, und vertheilen sich schliesslich vollständig im dem umgebenden Lösungsmittel.' The material in the central region is probably only preserved by the best fixing fluids.

One should notice in this connexion that Kurssanow (1909) described material of *N. lubricum* both with and without the pyrenoid. He believed the absence of the pyrenoid to be a sign of degeneration and approaching death of the cell, which can be artificially produced by fixing in the laboratory. He stated that material fixed in the field shows the pyrenoid, while material fixed in the laboratory shows none. He described a line of development from presence to absence of the pyrenoid which does not conform in the least with the mode of development in my material and seems to me very doubtful. Kurssanow used as fixing fluids iodine in sea-water and von Rath's fluid diluted ten times in sea-water. I obtained no difference in chromatophore structure between material fixed in the field and material fixed in the laboratory. Neither did I obtain any difference between the material fixed during the day and that fixed during the night. The nature of the fixing fluid used determined the only observed differences in the appearance of the chromatophore and pyrenoid.

There has been much diversity of opinion as to the nature of the pyrenoid and the methods by which it works. Schmitz (1882) described the pyrenoid as a rather homogeneous mass which in structure is closely allied to nuclein. Although actively concerned in the manufacture of starch, it takes no actual morphological part in this process. The starch is deposited in the clear zone surrounding the pyrenoid.

Meyer (1895) believed the pyrenoid to be crystalloidal in nature, serving merely as reserve food and taking no part in metabolism.

Schimper (1885) considered the pyrenoid to be of the nature of a protein crystal, but believed that it took part in the manufacture of starch in essentially the same manner as described by Schmitz.

Boubier (1899) likened the pyrenoid to a leucoplast and considered that not only the central body, but also the surrounding clear area, which

he described as penetrated by radiating granular strands, should be considered as the pyrenoid.

Timberlake (1901), working on *Hydrodictyon*, described its pyrenoid, which is protein in nature, as cutting off segments from itself in the form of concentric discs, which, by the deposition of starch, become transformed into starch grains.

Lutman (1910) has not been able to confirm Timberlake's results for *Closterium*. He found that the pyrenoid is not homogeneous, but is cut into by radiating clefts of lighter staining capacity. He considered it more probable that the cleavage resulting therefrom gives rise to new pyrenoids rather than to starch grains. All the starch grains, however, are formed around the pyrenoid.

McAllister (1914) described the chloroplast of *Anthoceros* as containing a large number of very small, distinct, proteid pyrenoid bodies, which probably increase in number by fission, the ones near the periphery of the chloroplast being transformed directly into starch grains.

Miss Bourquin (1917) has recently contradicted the earlier workers on *Zygnema*, by describing the formation of starch grains as taking place entirely independently of the pyrenoid, the latter apparently exerting no influence upon the process.

It will be seen, therefore, that there has been a wide difference in opinion with respect to the method by which the pyrenoid acts. Almost all workers are agreed, however, that it exerts some kind of an influence upon the development of starch, or, in other words, that it represents a photosynthetic centre.

There is no reason for believing that the pyrenoid of *Nemalion* is merely a mass of reserve food. We invariably find that wherever photosynthesis is most likely to occur, as at the periphery of the thallus, it is there that the pyrenoid is of largest size and of greatest prominence. On the other hand, all cells which are buried in the thallus, away from the more direct light, have a pyrenoid which is much reduced, or sometimes entirely wanting. If the central body were a mass of reserve food, it would be reasonable to suppose that it would be at least as plentiful in the inner as in the outer cells, for the food elaborated in the outer cells must be passed inward to all the living cells of the plant. The relative prominence of the pyrenoid seems rather to be correlated with photosynthetic activity. Besides this, tests show that the seat of reserve food is not in the chromatophore but in the surrounding cytoplasm of the cell.

The pyrenoid of *Nemalion* resembles those of *Hydrodictyon* and *Anthoceros* in being protein in nature, staining yellow with the xanthoproteic test, and, with the safranin and gentian-violet stain, standing out as a clear refractive brilliant-red structure in the centre of the surrounding area, which stains a rather neutral blue with gentian-violet. There seems,

however, to be no resemblance between the methods of action of the pyrenoid of *Hydrodictyon* and *Anthoceros*, and that of *Nemalion*. Whatever may be true with reference to the action of the central body in *Nemalion*, this much is certain, there is no segmentation or cleavage of the body to form starch grains. Having once attained its mature state it remains constant in size and appearance and never gives rise to any bodies. In other words, as Schmitz stated, it takes no actual morphological part in the process of starch formation. The fact, however, that we do not have in *Nemalion* a pyrenoid of the nature described by Timberlake and McAllister does not mean that it takes no part in photosynthesis. Starch grains are never formed in this plant through any agency, although reserve food is actively elaborated.

I have made some tests, both on living and fixed material, for the purpose of ascertaining the position and nature of the reserve food substances. Tests were made with a saturated aqueous solution of iodine by allowing it to filter slowly under a cover-slip, the material being mounted in water and under observation with the immersion lens. Diffused through the cytoplasm of those cells which were actively elaborating, there appeared a pale pink colour, which deepened to a brilliant wine-red, and then to an almost violet tone. The outer portion of the chromatophore became a brilliant saffron-yellow and the pyrenoid a dark blue-green. As the iodine became stronger, the whole contents of the cell stained a deep brown, completely disguising the colours first obtained. In certain cells this pinkish colour did not appear, namely, in the trichomes, many terminal cells of filaments, carpogonia, and the stalk-cells of developing cystocarps. In all of these cells chromatophores are either rudimentary or absent. The cells of the gonimoblastic filaments showed a small quantity of this material diffused through the cytoplasm, the amount increasing with the development of the chromatophore. Ungerminated carpospores gave quite a strong reaction which gradually diminished as germination progressed. Young germ-tubes failed to show the reaction.

Other material was treated with chloral hydrate-iodine solution in the same way. This in a more striking manner than before showed the brilliant red to violet coloration, and strengthening the solution did not bring on the undesirable brown staining with the iodine to so marked a degree. With this substance it was possible to see more clearly the small granules surrounding the pyrenoid, owing to the swelling action of the reagent. In all cases they took on a hue exactly like that of the pyrenoid. This colour was extremely difficult to classify, even in sectioned material, when unmodified by the surrounding yellow-staining portion of the chromatophore, because the granules are extremely small, and both they and the pyrenoid so refractive as to make a correct interpretation of the colour impossible. Since they appear to give exactly the same reaction as

the pyrenoid itself, I would consider that they bear no relation to starch grains. It appears from these tests that reserve food is not stored in the chromatophore, but is diffused through the cytoplasm of the cell.

This wine-red or violet-coloured reserve food material is the substance that has been termed 'Floridean starch'. Bruns (1894), Kolkvitz (1899), Bütschli (1903), and others have studied its presence in the red algae, and it appears to be an intermediate product between the starches found in most higher plants and the simpler di- and mono-saccharides. Bütschli describes it as intermediate between amyloerythrin and amyloporphyrin, giving violets, purples, reds, and red-browns with various reagents. He describes the colour obtained with iodine as wine-red to red-violet and with chloral hydrate-iodine as between violet and livid. In *Nemalion*, therefore, as in many other Florideae, we have as the main product of photosynthesis, not the complex starches of the higher plants, but a simpler starch, more soluble and giving different colour reactions with the various reagents. In view of the fact, therefore, that in *Nemalion* the reserve food substance is not built up into grains but is diffused throughout the cell, and that this substance is of a lower order than ordinary starch, we may conclude that, although the pyrenoid takes no morphological part in metabolism, as do pyrenoids in other groups, this fact does not disprove its metabolic function, and that it is actively concerned with the elaboration of Floridean starch.

Spermatogenesis.

The account which Wolfe has given of the development of the antheridial branch is in all points correct and needs not to be repeated. The cells are very small, averaging 3·5 μ in diameter, and have well-marked chromatophores, with a distinct darkly-staining central region at a stage corresponding to Fig. 8, *f*. The nucleus divides in the manner typical for all vegetative cells. The spindle is exceedingly small (Fig. 16), and usually each pole shows quite distinctly a minute black body at its tip. These figures are very plentiful, but are too small, 1·4 × 2·1 μ, to be favourable for a chromosome count.

Antheridia are budded off laterally from the cells of the antheridial branches. This process is accompanied by chromatophore as well as nuclear division (Figs. 16, 17), so that the young antheridium possesses a chromatophore, as described by Wolfe. Kylin (1916 *c*) did not observe the presence of a chromatophore, although admitting the possibility of such being present. After the antheridium is cut off, the chromatophore, which is of the most rudimentary nature, disappears from view. Whether it is still present and hid by the dense cytoplasm of the cell or whether it actually loses its identity could not be determined.

The process which Wolfe described as taking place during the maturation of the antheridium, by which the chromatophore is broken up and the

contents distributed in the nucleus, I have not been able to confirm. Appearances which might lead to such an interpretation I have only found in poorly fixed material. In such material the main part of the cell contents, usually including the nucleus, is massed at the distal end of the cell. If destaining is not carried quite far enough, some of the haematoxylin will remain in this dense area while in the remainder of the cell it has become completely extracted. This corresponds to the condition interpreted by Wolfe as a broken-down chromatophore, the material from which becomes aggregated at the distal end of the cell, where at least part of it, he believed, passes into the nucleus. In perfectly-fixed material there is no indication of such a structure at any stage, the cytoplasm remaining perfectly smooth and even throughout.

According to Wolfe, the food material thus brought into the nucleus rests for a time as a number of distinct granules in the nuclear cavity, but finally all passes into the nucleolus. When the spermatium escapes, the granules are still to be seen, although the nucleus itself is in a resting condition. Kylin considers that the granules are in reality eight to ten chromosomes, and that after a short resting period a true prophase is entered upon, the nucleus being in this condition at the time when the spermatium is set free. With the exception of chromosome count, the observations of Kylin are correct. The nucleus is at first at rest, but usually by the time when the spermatium escapes it has entered a prophase condition showing six to eight lightly staining chromosomes (Fig. 18).

In most of the red algae so far studied the spermatium nucleus is in prophase at the time of its escape. This is true of *Polysiphonia violacea* (Yamanouchi, 1906), *Rhodomela virgata* (Kylin, 1914), *Delesseria sanguinea* (Svedelius, 1914), *Scinaia furcellata* (Svedelius, 1915), *Griffithsia corallina* (Kylin, 1916 *a*), and *Bonnemaisonia asparagoides* (Kylin, 1916 *b*).

Several antheridial cells may be developed in succession from the same point on the antheridial branch, and it is not uncommon to see several empty antheridial walls one inside another with a new antheridium developing within.

One to several spermatia may fuse with a trichogyne (Figs. 20–24). At time of union the spermatium contains but one nucleus. Soon after fusion, however, the nucleus divides (Fig. 19), two male gamete nuclei being thus formed, both of which may pass into the trichogyne (Fig. 21). Wolfe and Kylin have both described and figured this division, but Kurssanow (1909) failed to find it. Division of the spermatium nucleus has been considered doubtful for *Griffithsia Bornetiana* by Lewis (1909), and is reported not to take place in *Polysiphonia*, *Scinaia*, or *Griffithsia corallina*. The presence of this division clearly shows, as Wolfe has pointed out, that the spermatium is, strictly speaking, the homologue of an antheridium.

Only one male nucleus succeeds in entering the carpogonium, although

others may get part way down the trichogyne (Fig. 22). At the time of passage through the trichogyne the male nucleus contains a large homogeneous nucleole, and is therefore in a resting condition (Figs. 20–23). In those forms in which the male nucleus is in prophase at the time of escape of the spermatium, and in which no division takes place, the male nucleus is reported to pass down the trichogyne in prophase, as a group of small bodies, each a chromosome. This is the condition described for *Polysiphonia*, *Griffithsia corallina*, and *Scinaia*.

After the male nucleus has passed into the carpogonium, the trichogyne is cut off from the carpogonium by a cleavage plane (Fig. 32), and later by a cell-wall (Figs. 21, 22, 24, 31). The supernumerary male nuclei therefore are left behind in the trichogyne (Figs. 21, 22), which remains for some time after fertilization, but finally withers and disappears (Text-fig. 3).

Oogenesis.

The procarp arises laterally from near the base of a vegetative tuft. It is easily recognized even in the one-celled condition by its form, being broader and shorter than the vegetative branches. The mature branch most frequently consists of four cells, but five and three are not uncommon, and two- or six-celled procarps are occasionally seen. The cells of the procarp have nuclei of the usual type. Each cell also possesses a chromatophore. In the basal cell this generally has a form more or less typical for any interior vegetative cell. The chromatophore becomes less and less well developed, however, in the cells above, until in the carpogonium and sub-carpogonial cell all sign of its pyrenoid structure is gone and the chromatophore consists merely of a dense mass of material very often surrounded by a narrow ring (Fig. 29). The cells before the fertilization of the carpogonium are entirely free from the darkly-staining bodies which are so characteristic at a later stage.

After the procarp has attained its full length, the terminal cell or carpogonium gives rise at its tip to a swelling which elongates and develops into the trichogyne (Figs. 26–29). At the base of the carpogonium is found the nucleus, which is quite large and possesses a prominent nucleolus and a distinct light-staining reticulum. Above the nucleus lies the chromatophore. Occasionally the position of the nucleus and chromatophore is reversed (Figs. 26, 27).

I have given special attention to the question of the presence of a trichogyne nucleus. I have examined many hundreds of unfertilized carpogonia with their trichogynes in every stage of development, and in only a few instances have I found indications of such a body. In two of these cases an extra nucleus was very clearly shown (Figs. 27, 28), which of course would constitute the so-called trichogyne nucleus. It should be noted, however, that in both of these cases the extra nucleus is not in the

trichogyne, but in the carpogonium. These extra nuclei are extremely small, insignificant structures, and they very quickly break down (Fig. 29). I have not found a single case where a nucleus was even doubtfully present in the trichogyne itself. The fact that in many hundreds of carpogonia and trichogynes, in all stages of growth, only a few possible examples of an extra nucleus have been found, convinces me that the formation of a trichogyne nucleus in *Nemalion* is not an invariable occurrence. When it is present, this extra nucleus is diminutive and lives only a short time, not even long enough, in most cases at least, to reach the trichogyne. We probably have here a reduction from the condition where a trichogyne nucleus was regularly present, to a state where it is only occasionally found. It will be seen from the figures that the position of the carpogonial nucleus in the cell bears no relation to the formation of a trichogyne nucleus. In Fig. 28 the division seems to have taken place below the chromatophore; in Fig. 27, above it. The carpogonial nucleus seems often to rest in the upper part of the carpogonium, even when no mitosis occurs.

There has been some dispute as to the presence of the trichogyne nucleus in *Nemalion* and related forms. Wolfe was positive in his assertion of its presence, describing it as well marked and unmistakable. Kylin, while describing and figuring it, stated that it is very small and visible only with difficulty in very young trichogynes. Kurssanow denied its presence entirely in *Nemalion lubricum* and *Helminthora divaricata*. Davis (1896), working on *Batrachospermum*, was the first to describe a trichogyne nucleus, but Osterhout (1900) and Schmidle (1899) did not find it in this form. Svedelius (1915) reported it in *Scinaia furcellata* and Kylin (1916 *b*) in *Bonnemaisonia asparagoides*. In the tetraspore-bearing red algae the evidence for its presence seems to be very clear. It has been described in *Polysiphonia* as a large body which does not disintegrate until after the fertilization of the egg; in *Rhodomela virgata*, *Griffithsia corallina*, and *Delesseria sanguinea* as a structure which quickly disintegrates and disappears. Lewis has not determined the situation for *Griffithsia Bornetiana*, but the probabilities are that it conforms to the condition found in *G. corallina*.

More than one theory has been advanced concerning the origin and nature of the trichogyne and its nucleus. Davis (1896) regarded the trichogyne of *Batrachospermum* with its nucleus and reduced chromatophore as having a certain degree of independence as a separate cell. Yamanouchi (1906) admitted that the trichogyne has a certain degree of independence, due to its possession of a nucleus, and believed that the multicellular trichogynes found in the Laboulbeniales illustrate a further development of this independence. However, he considered the trichogyne to have been originally an outgrowth of the carpogonium, and the trichogyne nucleus to represent a second female gamete nucleus now functionless.

Wolfe concluded that the trichogyne 'instead of being a mere hair-like outgrowth from a cell is at first a cell in the strictest sense of the word, which only later is specially modified in connexion with the reproductive processes'.

One of the striking features of *Nemalion* is the similarity in appearance and method of development between the trichomes and the trichogyne. It might not seem unreasonable to suppose that the carpogonium originally, like any other terminal cell, possessed the capacity of budding off trichomes, and that, after the male gametes lost their power of motility, this hair was modified into a receptive organ, and the formation of a wall between it and the carpogonium suppressed. The trichogyne would then represent a separate cell which has in some measure lost its independence. Such an explanation, however, looks no farther than the species under consideration. When we consider *Nemalion* and other red algae in the light of evolutionary relationships and study resemblances to other forms, and especially to *Coleochaete*, another interpretation presents itself which is essentially that of Yamanouchi. The striking resemblance between *Coleochaete* and the red algae, in vegetative structure, reproductive organs, and the development of the fertilized egg, lends weight to the possibility that the trichogyne-like outgrowth of the oogonium of *Coleochaete* is homologous to the trichogyne of the red algae. By this interpretation, the trichogyne of *Nemalion* would not represent a separate cell, and the trichogyne nucleus is probably the vestige of a second female gamete.

Fertilization.

At the time the male nucleus enters the carpogonium, the female nucleus may lie at any position in that cell, being perhaps more often found in the upper part than in the lower. Both gamete nuclei at time of fusion are in the resting condition. As the nuclei come together, it may be seen that they are somewhat unequal in size (Figs. 31, 32), the female being about half as large again as the male. The process of fusion of the nuclei is a slow one, and stages are, therefore, often found. The nuclear membranes melt away at the point where they come together, and the contents of the male nucleus pass into the cavity of the female. The chromatic nucleoles then fuse (Fig. 24). If nuclear union has occurred in the upper part of the cell, the zygote nucleus may remain in that position until after the first mitosis, or it may migrate back to the lower part of the cell.

Reduction and Development of the Cystocarp.

The zygote nucleus increases in size and remains for some time in a resting condition, while an additional thickness of wall is laid down completely around the protoplast. In the resting state (Fig. 33) the zygote nucleus possesses a reticulum of exceedingly delicate, closely interwoven

threads. After a time, however (Fig. 34), the individual threads begin here and there to show signs of thickening, and it is soon evident that with this gradual thickening there is a corresponding decrease in the length of the reticulum, the meshes becoming larger in size and the threads fewer in number. This process is often attended by a knotting at the points where two strands cross (Fig. 35). As the threads thicken they become quite granular, with definite bodies scattered all along them. At this point the threads show parallel arrangements (Fig. 36), and it is probable that an association of the threads in pairs takes place. I have noted no marked contraction of the thread system comparable to the stage of synapsis or synizesis in higher plants. From now on, the threads of the spireme present a much more even, smooth, and striking appearance, becoming thicker, while the total length markedly decreases. The spireme is double its former thickness and possibly represents a fusion of the male and female chromatic elements in the nucleus (Fig. 37). At the intersections of the threads, thickenings begin to form as knot-like structures. These knots grow larger, and at the same time the portions of the thread system connecting them become more and more attenuate until finally it can be seen that they are breaking here and there as they become too thin to hold. The process is one of condensation of chromatic material to form individual chromosomes, accompanied of course by their separation. The final result of this process is a set of eight bodies which are rather oblong in shape and quite large, and almost entirely separated from one another (Fig. 38). These are bivalent chromosomes, the paired members of which are to be segregated by the first mitosis in the carpogonium. The two portions of these bivalent chromosomes then separate (Fig. 39), and the result is a set of eight pairs of single chromosomes standing apart in the nucleus and forming a very clear stage of diakinesis (Fig. 40). My observations are thus in accord with the account of Kylin for *Nemalion* and of Svedelius for *Scinaia* in placing chromosome reduction at the first division of the zygote nucleus.

Diakinesis seems to be of rather short duration. The paired chromosomes soon become arranged in the equatorial plate and a spindle is formed. Polar structures are developed during the later prophase stages prior to diakinesis, but are not easily seen since they appear merely as slightly denser areas in the cytoplasm. That they are distinct bodies, however, is shown by such conditions as are pictured in Fig. 41, in which a slight amount of shrinkage has occurred, leaving the polar structures separated from the surrounding cytoplasm. The details of spindle formation were not observed.

The metaphase of the first mitosis is intranuclear (Fig. 42), although the nuclear membrane breaks down very soon after the equatorial plate is formed. The nucleolus is present during metaphase as an empty-looking

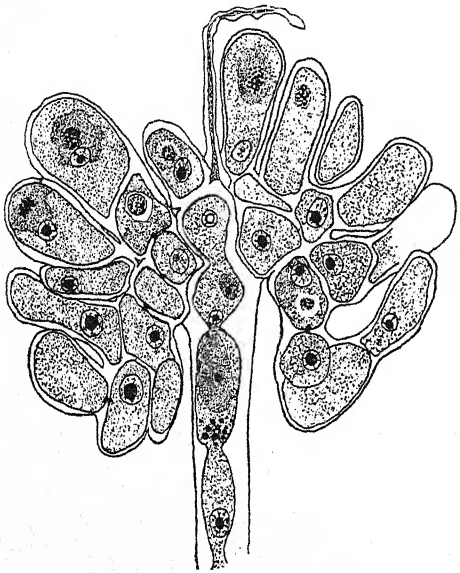
body inside the nuclear membrane and appears to be in process of disintegration. The polar structures are broad, lightly staining, and show no signs of an aster. At anaphase, the members of the eight pairs of chromosomes separate and pass as univalent chromosomes towards the poles of the spindle, where they become organized into the daughter nuclei. The individual chromosomes fuse into a large irregular body which rounds up and becomes a chromatin nucleolus. The nuclear membrane is formed and the nucleolus becomes suspended in the nuclear cavity by radiating fibrillae.

The two daughter nuclei thus formed lie one above the other in the now somewhat elongated carpogonium (Fig. 44). The chromatophore divides, one half passing upward, the other downward. A cell-wall is then cut in horizontally across the carpogonium, dividing it into an upper sporogenous and a lower hypogynous cell (Fig. 45). The hypogynous cell does not divide again, but the sporogenous cell gives rise to the gonimoblastic filaments of the cystocarp. We generally expect, in connexion with reduction phenomena, to find that both of the nuclei formed as a result of the first or 'heterotypic' division divide again by what is known as the homotypic division, the end-product being a group of four reduced nuclei. In *Nemalion*, however, we find that the nucleus of the hypogynous cell as a rule remains undivided until disintegration finally takes place. Very exceptionally this nucleus does divide, and in one instance I have been able to confirm Wolfe's observation of a vertical division of the hypogynous cell, suggesting that originally the full tetrad of reduced nuclei was formed. Such a tetrad is formed in *Scinaia*, as reported by Svedelius, although only one nucleus of the four remains functional.

The nucleus of the sporogenous cell soon passes through a mitosis (Fig. 46), and a cell-wall is laid down vertically, cutting off a lateral segment. This process is repeated several times, with the result that a number of cells are cut off laterally around the central sporogenous cell (Figs. 47, 48). Each of these lateral cells gives rise to a gonimoblastic filament which becomes branched in the manner typical of vegetative growth. When mature, each filament consists of a series of three or four cells, the terminal one of which develops into a carpospore (Text-fig. 3). When the carpospore is ready to be shed, the wall ruptures at the distal end and the protoplast escapes, leaving the empty wall attached to the filament. The growth of a filament does not stop with the shedding of the first spore, but a new cell is budded off within the empty shell (Fig. 49), which matures into a spore and escapes, leaving a second empty shell. Proliferation, as described by Wolfe, may in this manner continue throughout the growing season. The spore when first shed is elliptical, but it soon rounds up.

All through the development of the gonimoblastic filament, the

chromatophore may be seen. At first it is nothing more than a body of somewhat denser consistency than the surrounding cytoplasm, and, in well-fixed material, is in contact with the cytoplasm. As growth proceeds it becomes more distinct, following the same line of development as described for vegetative filaments (Fig. 8). The escaping spore possesses a very distinct chromatophore, which is in the condition corresponding to Fig. 8, *f*.



TEXT-FIG. 3. A cystocarp developing its first carpospores, showing the withering trichogyne, condition of the chromatophore, the fusion of the stalk-cells, and food particles in the stalk. × 1056.

Immediately after fertilization, the chromatophores of the upper stalk-cells of the carpogenic branch begin to show signs of disintegration, and here and there in the cytoplasm of these cells appear a number of densely-staining granules of varying size, which have probably been correctly interpreted by Wolfe as masses of reserve food material (Fig. 46). As the cystocarp develops, this process spreads to the lower cells of the stalk and the amount of material appears greatly to increase in the upper cells. The cells of the stalk then begin to fuse together by a widening of the connexion between them. The process begins at the top and progresses downward, resulting in one large fused cell, containing much reserve food material and in which the nuclei and chromatophores gradually disintegrate (Text-fig. 3).

MITOSES IN THE CYSTOCARP.

It is in the developing gonimoblasts that the details of mitosis can best be studied in *Nemalion*. This is due to the relatively large size of the nuclei and to the rapid growth and consequent abundance of figures. Every mitosis from first to last can clearly be followed.

The first indication of prophase is an increasing prominence of the linin network of the nucleus and the development, where the threads join, of numerous small aggregations of material, giving to the thread system a knotted appearance (Fig. 51). These bodies gradually grow larger and more prominent (Figs. 52, 61), but the threads themselves never become very distinct. As the bodies increase in size, it is evident that they also become fewer in number (Figs. 53, 54, 62). The threads connecting

certain of the bodies seem to contract, bringing them together so that they fuse, and in this way the number is brought down to eight, each of which is a chromosome (Figs. 54, 62). The thread system appears to be gradually absorbed into the chromosomes and they finally lie free in the nucleoplasm. Each of the chromosomes can be assumed to have retained its autonomy throughout the resting period, and the history of prophase is merely one of condensation and separation.

The chromosomes then become arranged to form the equatorial plate (Figs. 55, 56, 63, 64, 65). The spindle is intranuclear, and the nuclear membrane remains intact until the beginning of anaphase, when it quickly breaks down. At metaphase large, broad polar structures are conspicuously present at the tips of the spindles. The most striking feature of the metaphase in the cystocarp is the retention of the nucleolus. Presenting a rather empty and disorganized appearance, it takes a position on the edge of the equatorial plate, outside of the spindle, and does not disappear until the breaking down of the nuclear membrane. Wolfe observed this condition, but interpreted it as an extrusion of chromatin material representing part of the nucleolus. The presence of the nucleolus at this stage seems to be a fairly constant feature in the cystocarp, but has not been observed in vegetative divisions, this constituting the only difference that I have noted between the cystocarpic and vegetative mitoses.

The chromosomes split at the plate, and the daughter chromosomes, eight in number, pass to the poles (Figs. 57, 58). The whole of the chromatin content passing to each pole is organized into a chromatin nucleolus, around which a nuclear membrane is formed (Figs. 59, 60).

The nucleus thus organized increases in size, and the nucleolus becomes suspended by several radiating fibrillae (Fig. 60). It can then be seen that material passes out of the nucleolus, and that this material becomes distributed in a definite reticulum characteristic of the typical resting nucleus. We note, therefore, that at first the nucleolus is truly a karyosome, containing the whole of the chromatin material of the nucleus. Later on, this chromatic material becomes gradually distributed as a network throughout the nucleus. Occasionally, during the resting period, the nucleolus divides into two daughter nucleoli, a condition which has been seen nowhere but in the cystocarp (Fig. 50).

The chromosome number in all of the divisions of the cystocarp is clearly eight. Since Wolfe, however, concluded that the reduction takes place with the development of the first-formed carpospore from each filament, I have given particular attention to the condition at that stage. As may be seen from Figs. 61–66, however, the prophase and metaphase of this division differ in no way from those of previous mitoses in the cystocarp and the chromosome number is the same. The cystocarp of *Nemalion*, therefore, is haploid throughout its history in agreement with that of *Scinaia*.

Germination of the Carpospore.

The germination of the carpospore has been briefly described by Lewis (1912 *a*), but as he did not consider details of cytological structure, I have taken up the matter from that standpoint. The following account is based entirely upon sectioned material.

The rounded carpospore possesses a chromatophore, in the condition corresponding to Fig. 8, *f*, and a nucleus which is in the resting state showing a closely woven reticulum (Fig. 66). Previous to germination, the chromatophore may divide (Fig. 67), although as a general rule it remains undivided. Germination is by means of a tube which is put forth as a papilla and attains varying lengths. Into this the contents of the spore pass, the chromatophore in advance of the nucleus. If the chromatophore has previously divided, one of the two remains behind in the cyst (Figs. 80, 82). During the growth of the germ tube, and the passage into it of the spore contents, the nucleus completes the early stages of prophase (Fig. 70). These correspond exactly to those described for the cystocarp, but are even more striking in appearance, due to the large size and clearness of the nucleus. By the time that the nucleus is in mid-prophase, it has taken up a position near, or at the entrance to, the tube (Figs. 71, 72). There it completes the stages preliminary to mitosis and there it divides. Late prophase consists in the condensation and amalgamation of the numerous chromatic bodies until eight chromosomes are formed (Figs. 71–73). During late prophase, the polar structures may often be seen, at first minute, then becoming larger and more prominent (Figs. 70, 72, 74). At this time the nucleoplasm becomes very dense and stains heavily, so that it is almost impossible to make out the stages just preliminary to metaphase. When first formed, the equatorial plate is almost obscured by this state of affairs. A little later, however, the figure comes out clearly and it is possible to see that there are approximately eight chromosomes (Figs. 77, 78). The spindle is intranuclear (Figs. 75, 78), and no trace of the nucleolus has been seen at metaphase. The figure may lie in a perpendicular or in a horizontal position with respect to the axis of the germ tube (Figs. 75–77). The daughter chromosomes separate (Figs. 78, 79), pass to the poles, and are organized in the usual way to form daughter nuclei. One of the daughter nuclei passes into the tube, the other remains behind in the cyst, and a wall is laid down, cutting the germ tube off from the cyst (Figs. 80, 81).

The subsequent history of the cyst is interesting. If the chromatophore has divided previous to germination, one of the daughter chromatophores remains in the cyst and soon begins to break down. The nucleus also in many cases disintegrates, but it may undergo a mitotic division. The figure is occasionally seen (Figs. 83, 84), and even prophase stages have been observed (Fig. 82). Following the mitosis, however, the daughter

nuclei do not become completely organized. The chromatic material is gathered together into nucleoli, but no nuclear membranes are formed and the two bodies lie side by side for some time before they finally disintegrate (Figs. 85–7). The appearance of these two bodies side by side is a very common one, and it is probable that this division takes place frequently. There is a considerable variation as to the time at which it occurs. It may take place very soon after the cyst has been cut off (Figs. 85, 86), or it may not occur until the filament is two-celled (Fig. 83). The presence of this division is very interesting since it recalls the second division of reduction mitoses, as reported in the germination of certain algal spores (e.g. *Spirogyra*); but the evidence is clear that this is not the point of reduction in *Nemalion*, and the mitosis may simply refer to a time when the basal cell of the sporeling retained vegetative activity, perhaps giving rise to additional filaments.

Meanwhile the germ tube has grown greatly in length and the tube nucleus has come to occupy a position lateral to the now quite long chromatophore (Fig. 89). Usually the chromatophore divides first (Figs. 81, 90), followed by the nucleus, but the reverse may be the case (Figs. 89, 92). The second mitosis varies in no particular from the first. After nuclear division a cross-wall is laid down, resulting in a two-celled filament. In the same manner the third mitosis takes place and the third cell is formed (Fig. 94). Miss Chester (1896) has followed the development of the sporeling through subsequent phases, until it assumes the appearance and method of growth of the typical *Nemalion* plant.

The events recorded in this paper seem clearly to indicate that there is no phase in the life-history of *Nemalion* corresponding to the sporophyte of other groups. The cystocarp is shown not to be fundamentally sporophytic in nature, but to correspond closely to the tissue development leading to zoospore formation in the fertilized oogonium of *Coleochaete*, where Allen (1905) has placed for this plant the point of chromosome reduction in the first mitosis of the fertilized egg.

Discussion.

The problem of alternation of generations in the red algae is a perplexing one, and would demand more space for a complete discussion than can here be given. It will, therefore, be but briefly considered.

We find in the Florideae two distinct divisions. The first group, termed 'haplobiontic' by Svedelius (1915) and represented by *Nemalion* and *Scinaia*, presents a life-history without a change of chromosome number in the cystocarp and without a tetrasporic phase. The second group, termed 'diplobiontic', carries a diploid number of chromosomes through the gonimoblasts of the cystocarp, through the carpospore, and through

a tetrasporic generation. A striking feature of the tetrasporic plant, in almost every case, is the fact that morphologically it is almost identical with the sexual plant. *Galaxaura* excepted (Howe, 1917), there have been reported no morphological differences between these two generations that cannot be correlated with the peculiarities of their reproductive organs ; and even in *Galaxaura* the differences between sexual and tetrasporic plants are small and not phylogenetically fundamental. There are no forms in which the tetrasporic generation appears to be in process of development from something simpler to something more complex. In other words, there is no evidence in the red algae pointing to a separate origin and gradual development in complexity of the tetrasporic generation comparable to the increasing complexity of the alternating sporophytic generation in the Bryophytes and Pteridophytes.

Evolution in the red algae has probably been somewhat as follows. All of the red algae at one time were without tetraspores or cytological alternation of generations. They were all in agreement in the fact that reduction occurred at the first division of the zygote. Although none possessed tetraspores, some of them bore monospores, just as some of the non-tetrasporic plants to-day possess monospores. The present-day tetrasporic plants came about through a postponement of the time of reduction, at first perhaps to the germination of the carpospore and finally to the following generation. The failure of reduction to occur at carpospore germination meant that the next generation was diploid instead of haploid, as it otherwise would have been. Original monospores then probably became the seat of chromosome reduction, developing four tetraspores by the cutting in of walls following the reduction divisions. The occasional appearance of abnormal or partly developed tetraspores on sexual plants indicates a close relationship between the monosporangium and the tetrasporangium, suggesting strongly a homology. All of the evidence so far seems to point toward this as the course of evolution in the Florideae. The morphological similarity of the sexual and tetrasporic plants, the sharp break between haplobiontic and diplobiontic types, the presence of incomplete tetraspores on sexual plants and possibly apogamous sexual organs on tetrasporic plants, all seem to favour this view. Cytological investigation is much to be desired on other forms of the haplobiontic group. It is possible that types may be found in which reduction takes place with the germination of the carpospore. Such forms would constitute an important link, clearly indicating the above as the probable course of evolution.

We have, therefore, a method of evolution in the Florideae very different from that found in the Archegoniates. The situation in the Archegoniates is simple. The sporophyte has been developed as an entirely new structure to bear the diploid phase. The sporophyte and the

diploid condition have arisen simultaneously, and both have become extended and amplified through the same cause—a progressive sterilization of sporogenous tissue. The sporophyte structure is antithetic with the gametophyte structure, as is the diploid with the haploid condition.

The situation in the Florideae, however, is more complex. In those forms which possess a sporophytic phase, the diploid condition is not borne by one sporophyte structure but by two structures—the cystocarp and the tetrasporic plant—neither of which in its origin is wholly analogous with the sporophyte of the Archegoniates. The cystocarp has arisen as a structure antithetic to the sexual plant, and has done so in the same way that the sporophyte of the Archegoniates has arisen, namely, by a sterilization of sporogenous tissue, having possibly come from a condition similar to that found in *Coleochaete*, where a number of cells (eight) are formed by division of the zygote, each of which, however, gives rise to a zoospore. By progressive sterilization in the cystocarp, a certain amount of tissue formation has come to precede spore formation. We have, therefore, in the cystocarp of a form like *Nemalion*, a structure which we may look upon as a spore-bearing epiphytic plant, alternating with the sexual plant and bearing an antithetic relation to it. The cystocarp, however, differs in one very fundamental respect from the sporophyte of the Archegoniates in that it has originated independently of the appearance of a diploid condition. In *Nemalion* and *Scinaia* the cystocarp is already well developed, although the diploid condition has as yet not appeared. The cystocarp, therefore, unlike the sporophyte of the Archegoniates in its origin, was not fundamentally sporophytic in nature.

The only structure in the red algae which is always diploid in character is the tetrasporic plant. This, as we have already pointed out, is morphologically identical with a sexual plant, but is modified by the possession of a diploid number of chromosomes. It resembles the sporophyte of the Archegoniates in that it carries the double number of chromosomes, but in origin is entirely different from this, since it is not an entirely new structure, but only an old one made over.

We have, therefore, two markedly different situations. On the one hand, in the Archegoniates we find a sporophyte appearing simultaneously with the diploid condition and representing something as new, morphologically, as is the diploid condition cytologically—a structure without a phylogenetic ancestry. On the other hand, in certain red algae we have a sporophytic phase composed of two separate generations, one of which (the cystocarp) is antithetic to the sexual plant, but was in existence before the diploid condition made its appearance; the other of which (the tetrasporic plant) is the morphological homologue of the sexual plant. In the Archegoniates two antithetically alternating plants correspond to two antithetically alternating cytological phases. In the red algae these

cytological phases are borne by three distinct generations, two of which are morphologically homologous, while the third is morphologically antithetic to the other two.

Two terms have been widely applied in treating of the subject of alternation of generation. These terms are 'antithetic' and 'homologous'. As far as cytological alternation is concerned, it may be stated as axiomatic that the haploid and diploid phases in all plants bear an antithetic relation the one to the other. The diploid condition is new, and in no way homologous to the haploid condition.

Since the term 'alternation of generations', however, includes not only cytological but also morphological alternation, the application of either term to Floridean alternation of generations is unsatisfactory, since neither expresses the whole truth with reference to the situation in the red algae. The great confusion which has arisen over the subject has been largely a result of attempting to apply terms coined to express the relatively simple conditions in the Archegoniates to the more complex situation in the red algae.

It is not my purpose to offer a new terminology applying to this situation, but to point out the great need of such. While we may continue for the present to use the term 'antithetic' as expressing a larger percentage of the whole truth than does the term 'homologous', yet we should bear in mind that the situation in the red algae is not strictly comparable to that in the Archegoniates. It is to be hoped that in the near future a term will be proposed which will be expressive of the whole truth with reference to the morphological and cytological nature of the alternation of generations in the red algae.

Summary.

1. *Nemalion* possesses a true pyrenoid, which appears as a densely-staining body in the centre of a radiating chromatophore, its prominence being directly proportional to its opportunities for photosynthetic activity. This pyrenoid is actively concerned in the elaboration of soluble 'Floridean starch', which stains wine-red to violet with iodine, and lies diffused throughout the cytoplasm of the cell.

2. The nucleus of the spermatium is in prophase of mitosis at the time of the escape of the spermatium and divides after the latter has become attached to the trichogyne. Hence the spermatium is the homologue of an antheridium.

3. Several male nuclei may pass into the trichogyne, but only one enters the carpogonium.

4. A trichogyne nucleus is only occasionally formed, and, when present, breaks down very quickly.

5. The fusion of gamete nuclei involves a fusion of the chromatic nucleoles.

6. Chromosome reduction occurs with the first division of the zygote nucleus. The threads of the delicate reticulum thicken and shorten and finally take on a parallel arrangement. A fusion of threads then apparently takes place, followed by condensation of material to form eight bivalent chromosomes. These then become differentiated as eight pairs of chromosomes distributed about the nuclear cavity in a clear stage of diakinesis. The sixteen chromosomes of these eight pairs are segregated by the first mitosis, which is therefore a reduction division.

7. The chromosome number is eight throughout all nuclear divisions in the history of the plant, except the first mitosis of the zygote nucleus.

8. Vegetative mitoses are best studied in the cells of the developing cystocarp. The resting nucleus contains a large nucleole and a reticulum of delicate threads. Approaching mitosis is indicated by the appearance where the threads cross of numerous granules which, increasing in size, are brought together by the contraction of the threads, fusing successively until the eight chromosomes are differentiated. The spindle is intranuclear, and conspicuous polar structures are present. The nucleolus, presenting an empty appearance, takes a position during metaphase on the edge of the equatorial plate, outside of the spindle, and apparently disappears with the breaking down of the nuclear membrane. The two sets of daughter chromosomes during telophase severally organize a chromatin nucleole from which material is later distributed as a network throughout the resting nucleus.

9. The nucleus left in the carpospore cyst after the first division of the carpospore nucleus may again divide, but the resulting nuclei do not become completely organized and soon break down.

10. Since reduction, as in *Scinaia* and *Coleochaete*, takes place with the first mitosis of the zygote nucleus, the cystocarp of *Nemalion* is not sporophytic in character, and there is no cytological alternation of generations in this plant.

UNIVERSITY OF PENNSYLVANIA,
July, 1918.

LITERATURE CITED.

ALLEN, C. E. (1905): Die Keimung der Zygoten bei *Coleochaete*. Ber. deut. bot. Gesell., xxiii. 286.

BOUBIER, A. M. (1899): Contributions à l'étude du pyrénoïde. I. La membrane pyrénoïdienne. Bull. Herb. Boiss., vii. 451–8.

BOURQUIN, HELEN (1917): Starch Formation in *Zygnema*. Bot. Gaz., lxiv. 426–34.

BRUNS, E. (1894): Ueber die Inhaltskörper der Meeresalgen. Flora, lxxix, Erg.-bd., pp. 159–78.

BÜTSCHLI, O. (1908): Notiz über die sogenannte Florideenstärke. Verhandl. d. Heidelb. Naturhist.-Med. Vereins, N. F., vii. 519–28.
CHESTER, GRACE D. (1896): Notes concerning the Development of *Nemalion multifidum*. Bot. Gaz., xxi. 340–7.
DAVIS, B. M. (1896): The Fertilization of *Batrachospermum*. Ann. Bot., x. 49–76.
———— (1916): Life Histories in the Red Algae. Amer. Nat., l. 502–12.
HOWE, MARSHALL A. (1917): A Note on the Structural Dimorphism of Sexual and Tetrasporic Plants of *Galaxaura obtusata*. Bull. Torrey Bot. Club, xliii. 621–4.
KOLKVITZ, R. (1899): Beiträge zur Biologie der Florideen. Ber. deut. bot. Gesell., xvii.
KURSSANOW, L. (1909): Beiträge zur Cytologie der Florideen. Flora, xcix. 311–36.
KYLIN, H. (1914): Studien über die Entwicklungsgeschichte von *Rhodomela virgata*. Svensk Bot. Tidskr., viii. 33–69.
———— (1916 *a*): Die Entwicklungsgeschichte von *Griffithsia corallina*. Zeitschr. f. Bot., viii. 97–123.
———— (1916 *b*): Die Entwicklungsgeschichte und die systematische Stellung von *Bonnemaisonia asparagoides*. Zeitschr. f. Bot., viii. 545–86.
———— (1916 *c*): Ueber die Befruchtung und Reduktionsteilung bei *Nemalion multifidum*. Ber. deut. bot. Gesell., xxxiv. 257–71.
LEWIS, I. F. (1909): The Life-history of *Griffithsia Bornetiana*. Ann. Bot., xxiii. 639–90.
———— (1912 *a*): The Germination of the Spore of *Nemalion multifidum*. Science, xxxv. 154.
———— (1912 *b*): Alternation of Generations in certain Florideae. Bot. Gaz., liii. 236–42.
———— (1914): The Seasonal Life-cycle of some Red Algae at Woods Hole. Science, xxxix. 253.
LUTMAN, B. L. (1910): The Cell Structure of *Closterium Ehrenbergii* and *Closterium moniliferum*. Bot. Gaz., xlix. 241–55.
MCALLISTER, F. (1914): The Pyrenoid of *Anthoceros*. Amer. Journ. of Bot., i. 79–95.
MEYER, A. (1895): Untersuchungen über die Stärkekörner. Jena.
OSTERHOUT, W. J. V. (1900): Befruchtung bei *Batrachospermum*. Flora, lxxxvii. 109–15.
SCHIMPER, A. W. F. (1885): Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. wiss. Bot., xvi. 1–247.
SCHMIDLE, W. (1899): Einiges über die Befruchtung, Keimung und Haarinsertion von *Batrachospermum*. Bot. Zeit., lvii. 125–35.
SCHMITZ, Fr. (1882): Die Chromatophoren der Algen. Verh. des Nat. Ver. preuss. Rheinlande u. Westfalens.
SVEDELIUS, N. (1912): Ueber die Spermatienbildung bei *Delesseria sanguinea*. Svensk Bot. Tidskr., vi. 239–65.
———— (1914): Ueber die Cystocarpienbildung bei *Delesseria sanguinea*. Svensk Bot. Tidskr., viii. 1–32.
———— (1915): Zytologisch-entwicklungsgeschichtliche Studien über *Scinaia furcellata*. Nov. Ac. Reg. Soc. Scien. Upsal., Ser. IV, vol. iv.
TIMBERLAKE, H. G. (1901): Starch-formation in *Hydrodictyon utriculatum*. Ann. Bot., xv. 619–35.
WOLFE, J. J. (1904): Cytological Studies on *Nemalion*. Ann. Bot., xviii. 607–30.
YAMANOUCHI, S. (1906): The Life-history of *Polysiphonia*. Bot. Gaz., xlii. 401–49.

EXPLANATION OF PLATES XXII–XXIV.

Illustrating Mr. Cleland's paper on *Nemalion multifidum*, Ag.

All figures were drawn with the aid of a camera lucida at the level of the table, under a Spencer 1·8 mm. objective. The following were the oculars used, with the magnifications obtained: Zeiss compensating No. 12, approximately 2,200 diameters; Zeiss compensating No. 18, approximately 3,300 diameters; Spencer 10 ×, 1,530 diameters; Spencer 18 ×, 2,140 diameters.

PLATE XXII.

Vegetative Structure.

Fig. 1. Terminal cell at the apex of the thallus, nucleus in prophase. × 2,200.

Fig. 2. Same, nucleus in later stage of prophase. × 2,200.

Fig. 3. A cell at the apex of the thallus putting forth a bud which will develop into a side-branch. Metaphase of a vegetative mitosis, showing eight chromosomes. × 2,200.

Figs. 4–6. Stages in the division of a terminal cell, showing division of the chromatophore. × 1,520.

Fig. 7. Terminal cells of a central strand filament, with no sign of a chromatophore. × 2,200.

Fig. 8. Development of the chromatophore. *a*, earliest stage; *b*, a little later; *c*, *d*, *e*, development in an interior cell; *f*, *g*, *h*, development in a terminal cell.

Fig. 9. Mature chromatophore in a terminal cell. × 2,200.

Fig. 10. Mature chromatophore in a cell midway between the base and the apex of a filament. × 2,200.

Fig. 11. Mature chromatophore in a cell at the base of a filament. × 2,200.

Fig. 12. Mature chromatophore in a terminal cell, the pyrenoid divided, a frequent occurrence. × 1,520.

Fig. 13. Mature chromatophore in a terminal cell, showing the effect of Merkel's fluid, the pyrenoid broken down. × 2,200.

Figs. 14, 15. Mature chromatophore in a terminal cell, showing the effect of chromacetic acid, the pyrenoid partially or wholly destroyed. × 2,200.

Spermatogenesis.

Fig. 16. Metaphase at the cutting off of an antheridium. × 2,200.

Fig. 17. Telophase and chromatophore division at the cutting off of an antheridium. × 2,200.

Fig. 18. Prophase in the antheridium. × 3,300.

Fig. 19. Metaphase in a spermatium which is attached to a trichogyne. × 3,300.

Fig. 20. Male nucleus in the trichogyne. × 1,520.

Fig. 21. A fertilized carpogonium. Two spermatia are seen. A nucleus from the lower one has fertilized the egg, the other nucleus is disintegrating in the lower portion of the trichogyne. Both nuclei belonging to the upper spermatium are visible, one still within the spermatium, the other in the trichogyne. × 1,520.

Fig. 22. Male nuclei in the trichogyne. One has broken down. × 1,520.

Fig. 23. Male nucleus entering the carpogonium. × 1,520.

Fig. 24. Several spermatia on one trichogyne. Male and female chromatic nucleoles fusing in the zygote nucleus. The carpogonium already cut off from the trichogyne. × 1,520.

PLATE XXIII.

Oogenesis and Fertilization.

Fig. 25. A developing procarp. × 200.

Fig. 26. A young carpogonium and trichogyne. No trichogyne nucleus is present. It is rather unusual to find the nucleus above the chromatophore at this stage. × 2,200.

Figs. 27, 28. Trichogyne nuclei. Fig. 28 shows that the trichogyne nucleus can be cut off in the lower portion as well as in the upper part of the carpogonium. × 1,520.

Fig. 29. Entire procarp with immature trichogyne. A trichogyne nucleus is present in process of disintegration. × 1,520.

Fig. 30. The female nucleus above the chromatophore, as the male nucleus descends in the trichogyne. The more usual position. × 1,520.

Figs. 31, 32. Fertilization. It more usually occurs at the upper end of the carpogonium than in the lower portion. × 2,200.

The Reduction Division.

Fig. 33. Resting zygote nucleus with delicate chromatic reticulum. × 2,200.

Fig. 34. Early prophase. The thread system beginning to thicken and shorten. × 2,200.

Fig. 35. Here and there knots of chromatic material have appeared. × 2,200.

Fig. 36. The threads present a granular appearance and show parallelism, suggesting fusion. × 2,200.

Fig. 37. Thick spireme. Eight chromatic knots are beginning to form in the angles of the spireme, and will develop into the eight bivalent chromosomes. × 2,200.

Fig. 38. Eight bivalent chromosomes, almost free from one another. × 2,200.

Fig. 39. Bivalent chromosomes free, and now split, forming pairs of single chromosomes. × 2,200.

Fig. 40. Diakinesis. Seven pairs of univalent chromosomes shown. × 2,200.

Fig. 41. A zygote nucleus approaching mitosis with the polar structures made conspicuous through shrinkage of the surrounding cytoplasm. × 2,200.

Fig. 42. Metaphase of the first mitosis which is the reduction division. The nucleolus is still present. The spindle is intranuclear. × 2,200.

Fig. 43. Metaphase of the first mitosis. × 2,200.

Fig. 44. After the first mitosis and before the cross-wall is formed. × 2,200.

Fig. 45. Carpogonium divided into the sporogenous and hypogynous cells. × 2,200.

The Developing Cystocarp.

Fig. 46. Sporogenous cell above with metaphase of the second mitosis. Food particles in a stalk-cell. × 2,200.

Fig. 47. Metaphase in a gonimoblast initial. × 2,200.

Fig. 48. Young cystocarp. × 1,520.

Fig. 49. The proliferation of carpospore initials. New spores being developed in old cysts. × 1,520.

Mitosis in the Cystocarp.

Fig. 50. Resting nucleus with two nucleoli. Often seen in the cystocarp, but nowhere else. × 2,200.

Fig. 51. Beginning prophase. × 2,200.

Fig. 52. Later prophase. Many chromatic bodies. × 2,200.

Fig. 53. Still later prophase. The bodies become fewer by fusion. × 2,200.

Fig. 54. Late prophase, showing chromosomes. × 2,200.

Figs. 55, 56. Metaphases. Spindle intranuclear, with polar structures and nucleolus at the side of the equatorial plate. × 2,200.

Figs. 57, 58. Anaphases. × 2,200.

Fig. 59. Telophase. Spindle remnants still show. × 2,200.

Fig. 60. Newly organized nuclei. Chromatin nucleoles suspended by fibrillae. All of the chromatin is contained still within these nucleoles. × 2,200.

Figs. 61–5. Prophases and metaphase of the division which results in the cutting off of the first carpospore from a filament. Behaviour as in previous mitoses in the cystocarp (Figs. 51–6). × 2,200.

PLATE XXIV.

The Germination of the Carpospore.

Fig. 66. A carpospore, the chromatophore with well-developed pyrenoid. × 2,200.

Fig. 67. A carpospore in which the chromatophore has divided. × 2,200.

Fig. 68. The effect of weak chromacetic acid on the pyrenoid. × 2,200.

Fig. 69. Protrusion of the germ tube. Early prophase in the nucleus. × 2,140.

Fig. 70. Early prophase. Polar structures have appeared. × 2,200.

Figs. 71, 72. Mid-prophase. Many chromatic bodies. Polar structures shown in Fig. 72. × 2,200.

Fig. 73. Late prophase. The chromatic bodies have condensed in number to eight chromosomes. × 2,200.

Fig. 74. Just previous to metaphase. Chromacetic fixed. Note effect on pyrenoid. × 2,200.

Figs. 75, 76. Metaphases. Spindles lying at different angles, intranuclear. × 2,200.

Fig. 77. Metaphase. Chromacetic fixed. × 2,200.

Fig. 78. Anaphase. Chromacetic fixed. Note effect on pyrenoid. × 2,200.

Fig. 79. Anaphase. × 2,140.

Fig. 80. The tube is cut off, leaving a nucleus and chromatophore in the cyst. The division of the chromatophore took place previous to germination. × 2,140.

Fig. 81. Nucleus undivided in the cyst. Chromatophore in the tube divides before the nucleus. × 2,200.

Fig. 82. Prophase in the cyst nucleus. This section is cut obliquely so that only a small portion of the tube is shown. Such a striking prophase is not often seen in the cyst, and is probably due to the unusual amount of cytoplasm left behind. × 2,200.

Fig. 83. Metaphase in the cyst. × 2,200.

Fig. 84. Anaphase in the cyst. × 2,200.

Fig. 85. The nucleus in the cyst has divided, but the daughter nuclei have not become completely organized. × 2,200.

Fig. 86. The two unorganized daughter nuclei in the cyst. A very usual condition. × 2,200.

Fig. 87. Two unorganized nuclei and a chromatophore in the cyst. × 2,200.

Fig. 88. Prophase for mitosis in the first tube cell. × 2,200.

Fig. 89. Metaphase taking place in the tube before chromatophore division, polar structures. × 2,200.

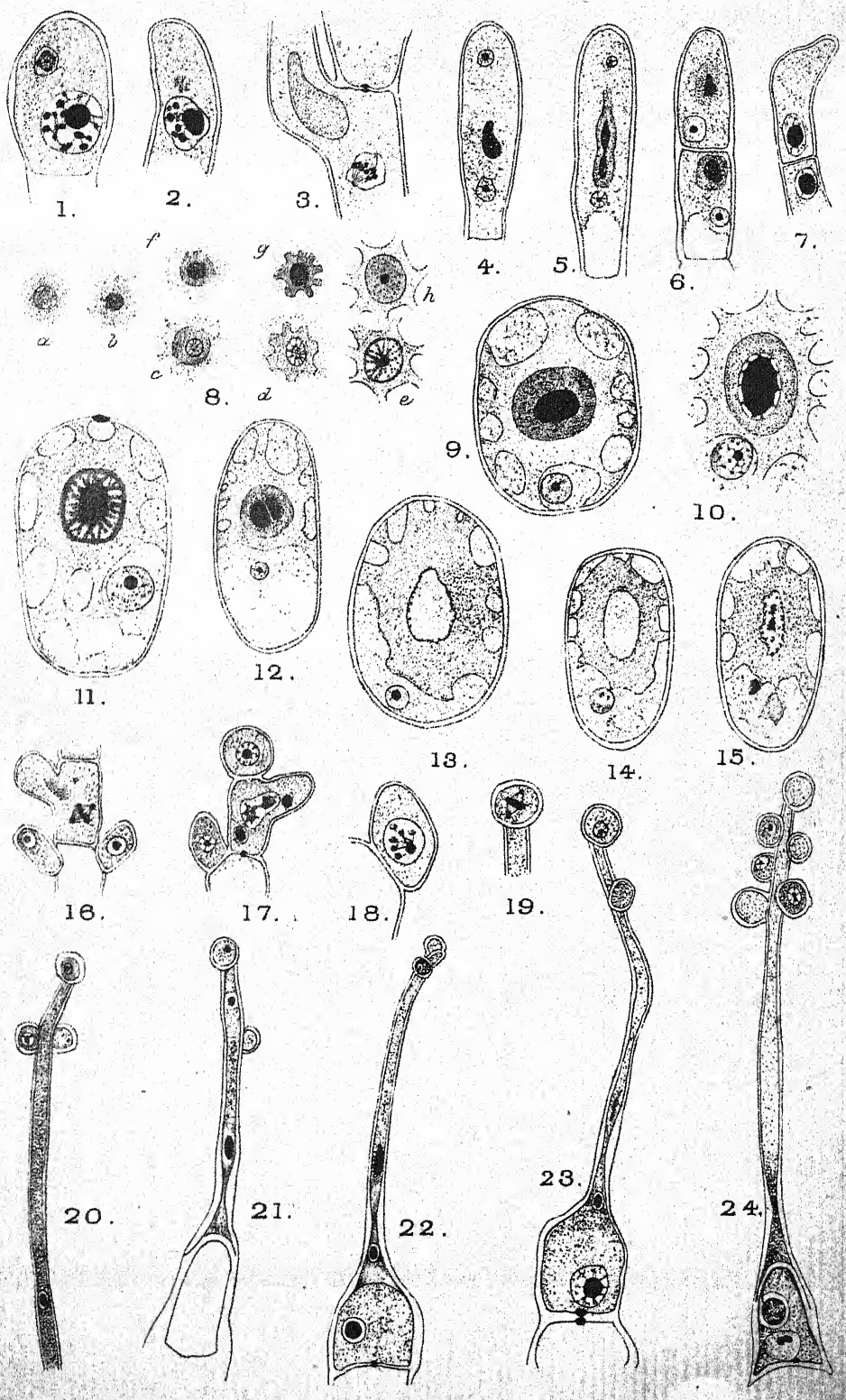
Fig. 90. Metaphase after chromatophore division. The more usual condition. × 2,200.

Fig. 91. Anaphase in the tube cell, polar structures. Transverse section of the tube. × 2,200.

Fig. 92. Telophase in the tube cell. × 2,200.

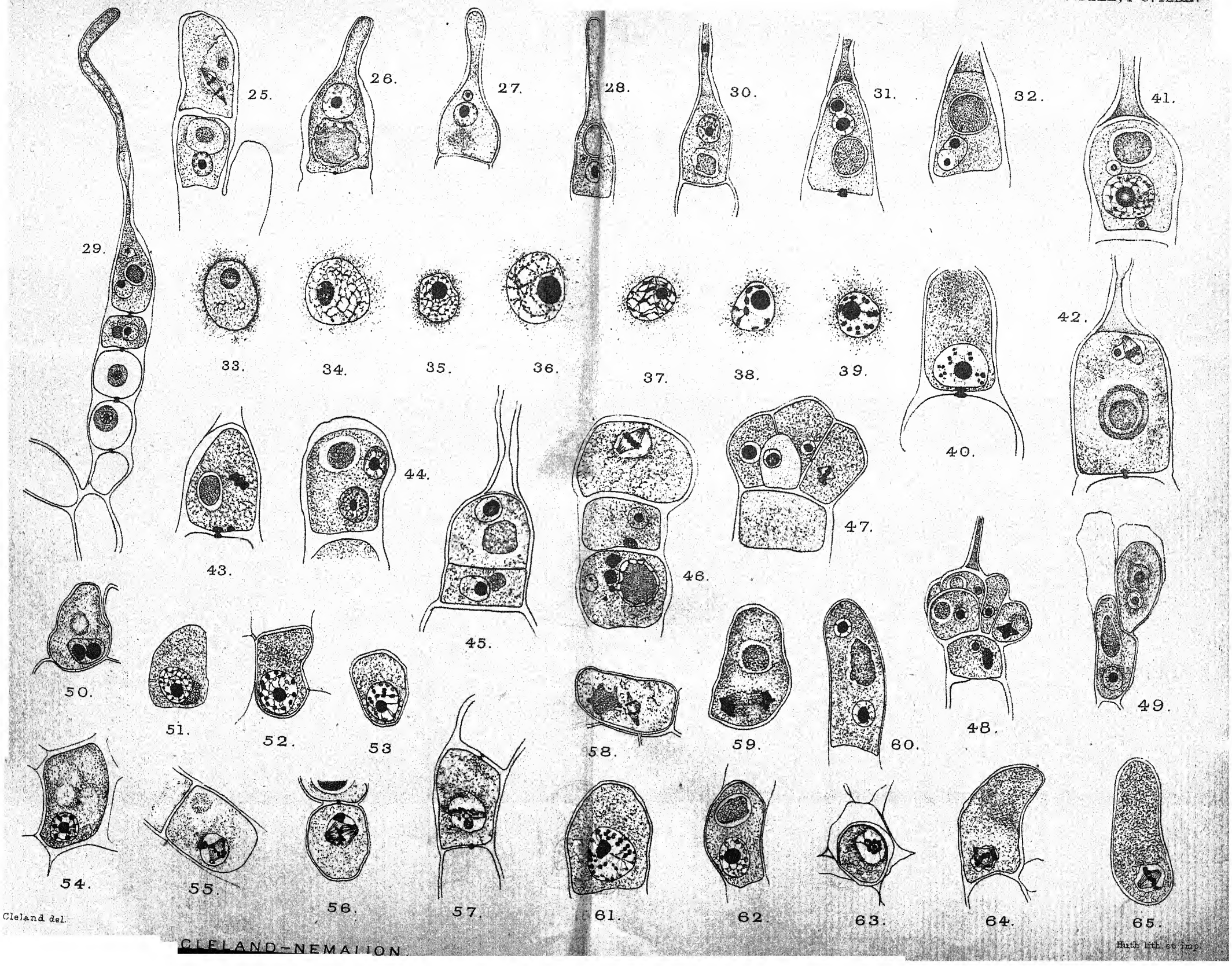
Fig. 93. Previous to wall formation. × 2,200.

Fig. 94. Two-celled stage of the tube. Third metaphase. × 2,200.



Huth lith. et imp.

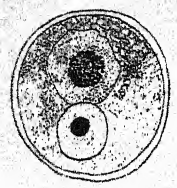
CLELAND — NEMALION.



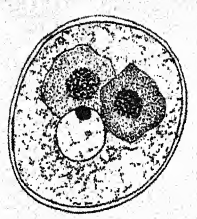
Cleland del.

CLELAND—NEMALION

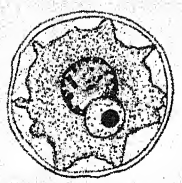
Huth lith. et imp.



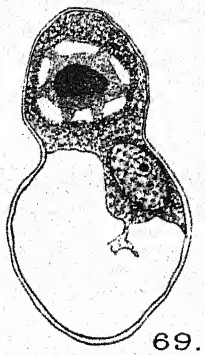
66.



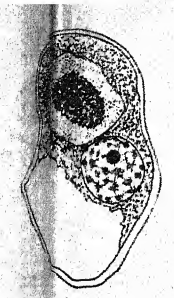
67.



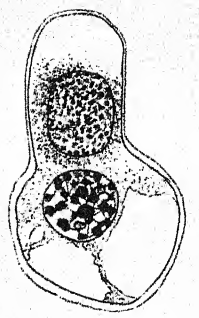
68.



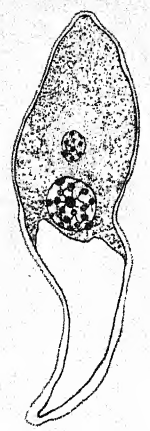
69.



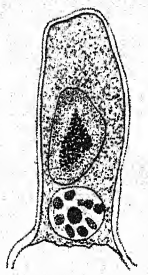
70.



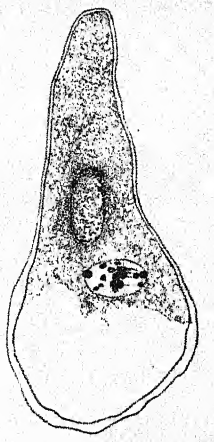
71.



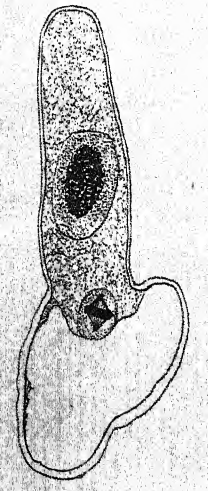
72.



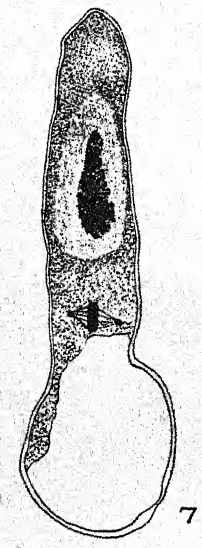
73.



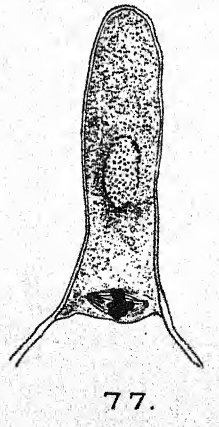
74.



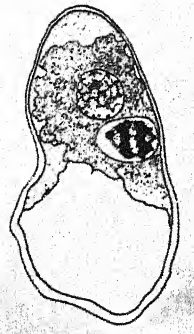
75.



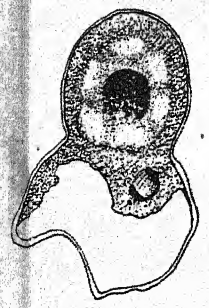
76.



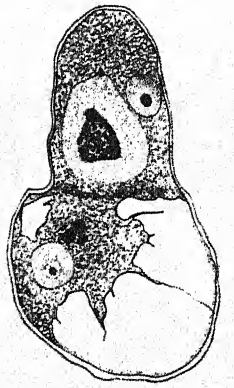
77.



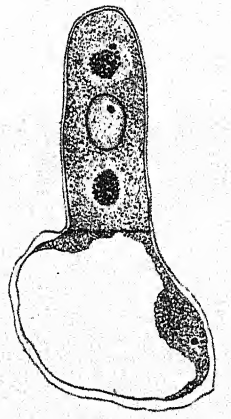
78.



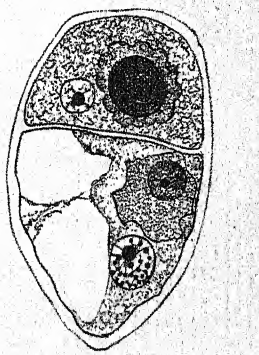
79.



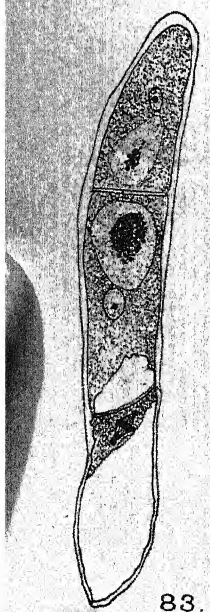
80.



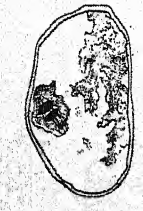
81.



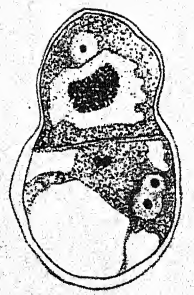
82.



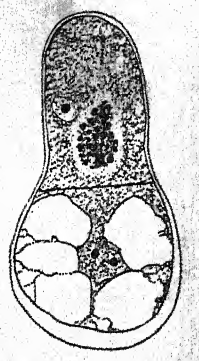
83.



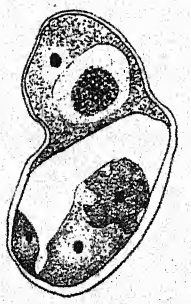
84.



85.

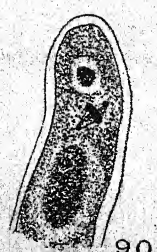


86.

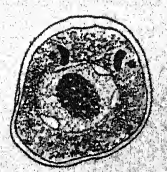


87.

89.

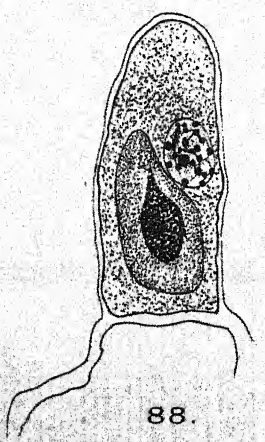


90.

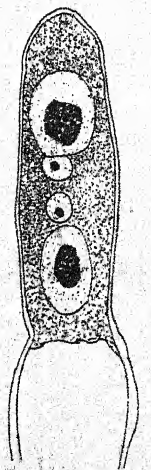


91.

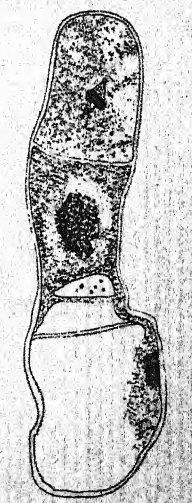
92.



88.



93.



94.

Cleland del.

CLELAND — NEMALION.

Huth lith et imp.

The Compound Interest Law and Plant Growth.

BY

V. H. BLACKMAN.

IN many phenomena of nature we find processes in which the rate of change of some quantity is proportional to the quantity itself. Since money put out at compound interest increases in this way—the rate of increase being clearly proportional to the amount of capital at any time—Lord Kelvin called the law which such processes follow 'the compound interest law'. The rate at which a body cools follows the compound interest law, for the hotter the body relative to its surroundings the more rapidly it loses heat. Again, the variation of atmospheric pressure with height above sea-level follows this law, as does also the velocity of a chemical reaction. Wilhelmy's law, discovered as long ago as 1850, that 'the amount of chemical change in a given time is directly proportional to the quantity of reacting substance present in the system', is simply a restatement of the compound interest law.

The importance of this law for the proper appreciation of the growth of a plant was brought home to the writer in 1917 in connexion with the results of some experiments on the growth of cucumbers carried out in association with Mr. F. Gregory at the Cheshunt Experimental Station.

It is clear that in the case of an ordinary plant the leaf area will increase as growth proceeds, and with increasing leaf area the rate of production of material by assimilation will also increase; this again will lead to a still more rapid growth, and thus to a greater leaf area and a greater production of assimilating material, and so on. If the rate of assimilation per unit area of leaf surface and the rate of respiration remain constant, and the size of the leaf system bears a constant relation to the dry weight of the whole plant, then the rate of production of new material, as measured by the dry weight, will be proportional to the size of the plant, i.e. the plant in its increase of dry weight will follow the compound interest law.

The fact that the increase in number of unicellular organisms, when not limited by external conditions, follows a regular geometric series has long been recognized. The resemblance also of the growth processes of animals and plants to an autocatalysis has been pointed out by a number of workers, as J. Loeb, W. Ostwald, Robertson, F. F. Blackman, Chodat

[Annals of Botany, Vol. XXXIII. No. CXXXI. July, 1919.]

and his pupils.[1] The application of the compound interest law to the growth of the higher plants, though of fundamental importance to a right understanding of the plant's rate of increase, has, however, been overlooked by most botanists,[2] and its recognition is sadly lacking in the text-books both of plant physiology and general botany. Chodat in his 'Principes de Botanique' (2nd edit., p. 133) appears to be the only text-book writer who even refers to the relation of growth to a geometric series, and his treatment of the subject appears under the section of the book which deals with the growth of the cell, and it is confined to pointing out the similarity of the growth of the cell to a process resembling autocatalysis.

Apart from any question of autocatalysis it is obvious that the increase in size of the assimilating surface of the young plant must constantly accelerate the rate of growth, and that the consideration of this acceleration is essential for the proper comparison of the final weight of different plants and of the same plants grown for different periods.

When money accumulates at compound interest, the final amount reached depends on (1) the capital originally employed, (2) the rate of interest, (3) the time during which the money accumulates. In the case of an annual plant the ultimate dry weight attained will depend on (1) the weight of the seed, since that determines the size of the seedling at the time that accumulation of new material begins; (2) on the rate at which the material present is employed to produce new material, i. e. the percentage increase of dry weight per day or week or other period; (3) the time during which the plant is increasing in weight.

It is clear then that some simple equation is required to relate these three factors to the final weight attained; such an equation does not appear to have been hitherto put forward by those few workers who have considered the growth relations of the whole plant from this aspect. Before dealing, however, with this attention may be drawn to the work of Noll and his pupils, who have provided the data of the growth relations of a number of plants during various stages.

Noll seems to have been the first to formulate the view that in the case of an annual plant the successive dry weights taken at regular periods follow a geometric series. In 1906 Noll read before the Niederrheinische Gesellschaft für Natur- und Heilkunde zu Bonn a paper (which appears only

[1] There can be no doubt that the development of an increasing number of rapidly enlarging cells which occurs in the development of most plant organs will cause a rapid acceleration of growth, producing a curve of growth which is very similar to that of an autocatalysed reaction. A process of autocatalytic nature does not, however, explain the rapid fall in the rate of growth in the later stages of development of the human body, or the fall in the rate of growth of a plant organ which has passed its 'grand period' of growth. The growth of an annual body of a plant organ of limited size is clearly dominated by factors other than those that play their part in a simple autocatalytic process.

[2] Since the above was written I have seen the proof of a paper (to appear in the Annals of Applied Biology) by Dr. F. Kidd and Dr. W. West in which they point out the importance of the compound interest law.

in title in the Proceedings of the society) entitled 'Über die Substanzquotienten pflanzlicher Entwickelungsstadien'. In the next two years three of his pupils, Gressler,[1] Hackenberg,[2] and Kiltz,[3] published inaugural dissertations dealing with the determination of the 'Substanzquotienten' of various plants.

From these papers it becomes clear that the 'Substanzquotient' is the factor which relates the dry weight at the end of any period of growth with the dry weight at the beginning of that period. Taking Gressler's results with *Helianthus uniflorus giganteus* we find that in five successive weeks the average dry weight in grammes of a number of plants was 0·0454, 0·147, 0·508, 1·653, 5·868, 17·33, 30·35, 46·2, 66·1, 88·9. Dividing the second by the first, the third by the second, and so on, we find that the successive weekly 'substance-quotients' were 3·24, 3·45, 3·24, 3·56, 3·0, 1·75, 1·52, 1·4, 1·3. The successive, weekly dry weights clearly exhibit at first a progression which is approximately geometrical; later, however, the rate of increase falls off, the series becoming an arithmetical one.

The 'substance-quotient' per week is obviously a clumsy and inaccurate method of expressing these results. What is required is some simple method of relating the plant's activity in the production of new material to time and to the initial weight of the seedling. Hackenberg and Kiltz merely state their results as 'substance-quotients' per week, but in calculating the average 'substance-quotient' over a period of many weeks Gressler treats his results as a discontinuous geometric series. The formula which would then apply is $W_1 = W_0 (1+r)^t$, where W_1 = the final weight, W_0 = the initial weight, r = the rate of interest, and t = time. Gressler's results for *Helianthus* calculated in this way per week and per day are shown in columns 5 and 6 of the accompanying table.

	Seedling weight. Grm.	*Final weight. Grm.*	*Time. Days.*	*Rate of Interest (Discontinuous).* *per week.*	*per day.*	*Rate of Interest (Continuous).* *per week.*	*per day.*
H. uniflorus giganteus	0·0327	17·33	37	227·6 %	18·5 %	119·0 %	17·00 %
H. nanus	0·0348	14·805	37	214·3 %	17·7 %	114·5 %	16·36 %
H. cucumerifolius nanus	0·00106	0·401	56	110·0 %	11·2 %	74·1 %	10·59 %
H. macrophyllus giganteus	0·0241	6·772	32	243·3 %	19·3 %	123·4 %	17·63 %
H. arboreus giganteus	0·0192	14·680	40	219·6 %	18·1 %	116·1 %	16·59 %

Treatment of the results in this way would, however, only be satisfactory if the additional material were added *discontinuously* at the end of each day or week. It is obvious, however, that during the daylight period the plant is adding new material continuously, and during rapid growth the plant

[1] P. Gressler: Ueber die Substanzquotienten von *Helianthus Annuus*. Inaug. Diss., Bonn, 1–29, Tables I–V, 1907.

[2] H. Hackenberg: Ueber die Substanzquotienten von *Cannabis sativa* und *Cannabis gigantea*. Inaug. Diss., Bonn, 1–27, 1908. Also Beihefte zum Bot. Centralbl., xxiv, pp. 45–64, 1908.

[3] H. Kiltz: Versuche über den Substanzquotienten beim Tabak und den Einfluss von Lithium auf dessen Wachsthum. Inaug. Diss., Bonn, 1908 (seen only in abstract, Bot. Centralbl., cx, p. 455, 1909).

is continuously, or nearly continuously, unfolding its leaves and increasing its assimilating area. The plant's increase is thus comparable rather to money accumulating at compound interest, in which the interest is added to the principal not daily or weekly, but continuously. The simple equation which best applies to the growth of active annual plants is thus:

$$W_1 = W_0 e^{rt},$$

where, as before, W_1 = the final weight, W_0 = the initial weight, r = the rate of interest, and t = time, and e is the base of natural logarithms.[1] Some of Gressler's results have been calculated on this basis of continuous addition at compound interest, and are given in the last two columns of the table. The rate of interest required to give the same final dry weight is naturally less when it is added continuously than when it is assumed to be added discontinuously, and far less than the rate of interest per week calculated from the weekly 'substance-quotients'.

As has already been stated, it is obvious from general considerations, and also from the equation, that the final weight attained will depend on the initial weight, the rate of interest (r), and the time. The differences in the dry weight attained by two plants may thus depend on simply the initial dry weights of the seedlings; if the rate of interest is the same the final weights will then vary *directly* as the initial weights. This shows the marked effect which large seeds as compared with small seeds may have on the final weight attained. Again, if the initial weights are the same a small difference in the rate of interest (r) will soon make a marked difference in the total yield, and the difference will increase with the lengthening of the period of growth. A difference of 1 per cent. in the rate of interest will in a period of 69 days double the final weight attained.

Oats in water culture may, according to Wolff, attain a dry weight 2,359 times that of the seed. If the growing period be taken as 100 days, the rate of interest on the basis of continuous addition is 7·76 per cent. per day. If the rate of assimilation per unit area should rise by 5·8 per cent. then, allowing 10 per cent. for loss of respiration, the final weight at the end of 100 days would go up 50 per cent. Plants of *Helianthus macrophyllus giganteus* (investigated by Gressler) with a seed weight of 0·0241 grm. may in 32 days reach a dry weight of 6·77 grm., i.e. a weight 251 times that of the seed. This on calculation by the equation given above requires that r shall be 0·1763 (i. e. an average rate of 17·63 per cent.) per day. An increase

[1] This formula can be expressed as $\log_e \frac{W_1}{W_0} = rt$. In using the formula it is only necessary to find the number which expresses the relation between the final and initial dry weights of the plant; and then to find the napierian logarithm ($\log_e$) of that number, or to find the common logarithm and multiply by 2·3026. The logarithm so found when divided by the time gives the rate of interest required. Suppose a plant has doubled itself in ten days. We find that the $\log_e 2$ is 0·69315; therefore the plant has been producing new material at the rate of 0·0693 (i. e. 6·93 per cent.) per day. If the period were 5 days the rate would be 13·8 per cent. per day; if 100 days, then 0·69 per cent. per day.

of assimilation of 2 per cent. would in this case increase the weight at the end of 32 days by about 20 per cent.

A marked difference in the rate of interest (r) is exhibited by different plants. The table given shows that in some species of *Helianthus* it may reach 17·6 per cent. per day, while in *H. cucumerifolius nanus* it is only 10·42 per cent. per day. In some results obtained by Stefanowska[1] with Maize in water culture the plants increased their fresh weight 27·5 times in 45 days; the rate of interest was therefore only 7·45 per cent. per day. Obviously some plants can work with far greater economy than others. Thus for every 100 grm. of dry material already present *H. macrophyllus giganteus* can produce new material at the average rate of 10·4 grm. per day; calculating from Hackenberg's results, we find that *Cannabis gigantea* may work at a rate of 13·1 per cent. per day for a short time, and (from Kiltz) that *Nicotiana Tabacum* may work at the rate of 20·5 per cent. per day. *Zea Mais*, on the other hand, as stated above, under some conditions works at the average rate of only 7·45 per cent. per day.

The rate of interest (r of the equation) is clearly a very important physiological constant. *It represents the efficiency of the plant as a producer of new material*, and gives a measure of the plant's economy in working. The rate of interest, r, may be termed the *efficiency index* of dry weight production, since not only does it indicate the plant's growth efficiency as measured by increase of dry material, but it also appears as an exponential term in the equation which expresses the relation between the initial dry weight, the final dry weight, and the period of growth. It may also be termed the 'economy constant' of the plant; it is of course comparable to the velocity constant of a chemical reaction.

It is suggested that in all water cultures, pot experiments, and similar experiments where dry weights are determined after a period of growth, the efficiency index should be calculated from the seed weight and the final weight attained, so that a measure of the plant's *average* economy of working may be obtained.[2] Such a calculation will show how far a large final weight is determined by a large initial weight or by a high efficiency index.

A glance at the table given above shows that the 'dwarfness' of *Helianthus cucumerifolius nanus* is due not only to the very small seed weight but also to the comparatively low efficiency of the plant, the efficiency index being only 0·1042 (or 10·42 per cent.) per day. This form of *Helianthus* is handicapped by a seed 1/300th of the weight of that of *H. uniflorus giganteus*, so that even if it had the same efficiency it could only attain 1/300 of the final weight of the latter. Other things being equal, a small seed is a permanent handicap to a plant in the production of material. *H. cucumerifolius nanus*, in order to attain after 37 days the same

[1] Comptes rendus de l'Acad. des Sciences, cxxxviii, 304–6, 1904.

[2] Where root parts are not available, the efficiency index can be given for the aerial parts only. The seeds should, where possible, be weighed without the testa.

weight as *H. uniflorus*, would have to work at an efficiency of 0·2621 (i. e. 26·21 per cent.) per day; with this high economy of working the plant would double its weight in less than three days. *For the highest production of vegetative material by the single plant two factors are necessary—large seeds, and a high economy in working represented by a large efficiency index.*

The importance of these two factors in breeding cereal crop plants should be borne in mind; it may be possible to breed for a high efficiency index. In many crop plants the matter is of course complicated by the effect of crowding on the efficiency of the individual plant, a question which requires further analyses.

The growth is naturally affected by external conditions, being higher when conditions are favourable, but even under the same conditions there are large variations in the economy of working of different plants, so the efficiency index is certainly to a large extent a characteristic of different species and varieties. It would be of great interest to determine to what these differences in efficiency are due. They may be the result of differences in the rate of assimilation per unit area of leaf surface, of differences in the rate of respiration, of differences in the thickness of the leaves, or of differences in the distribution of material to leaves on the one hand and to the axis on the other. The larger the proportion of new material that the plant can utilize in leaf production the greater, other things being equal, should be its efficiency.

It is clear, from Gressler's and Hackenberg's results with *Helianthus* and *Cannabis*, that the efficiency of the plant is greatest at first and then falls somewhat, but the fall is only slight until the formation of the inflorescence, when there is a marked diminution in the efficency index. For *Helianthus arboreus giganteus*, for example, the 'substance-quotients' for successive weeks are 3·11, 3·49, 3·71, 3·06, 2·59, 3·03, 2·0, 1·5, 1·4, 1·1, 1·3, 1·3. The sudden fall from 3·03 to 2·0 is associated with the appearance of the inflorescence.

The observations of Gressler, Hackenberg, &c., require repetition, for they worked with only a small number of plants and they give no idea of the experimental errors involved, though the differences in dry weight of the individual plants must have been considerable. It would be very valuable to have data for various agricultural and horticultural plants, showing the average efficiency index under various conditions. If the dry weight measurements were combined with a measurement of leaf area and leaf weight some insight could be obtained into the nature of the differences which exhibit themselves as differences in the efficiency index of different plants and as differences in the efficiency index of the same plant at different stages.[1]

[1] If such observations were combined with an estimation of the carbon content of the root, stem, and leaf, together with some measure of the rate of respiration, the analysis could be carried still further. It is hoped to undertake this work with some agricultural plants.

The fall in efficiency after the first few weeks of growth may perhaps be correlated with the mechanical relations connected with larger size. A doubling of the leaf area would require a stem of more than twice the weight to attain equal strength. Gregory[1] found that with larger leaf areas the ratio of stem weight to leaf weight went up.

As the efficiency of the plant is highest in its early stages favourable conditions at that time should have a marked effect on growth. If a plant, owing to such conditions, should double its size as compared with another plant, then there is no reason why that advantage should not be retained to the end of the growing season. A 'good start' means, among other things, a larger capital to work with throughout the growing season.

Gericke,[2] investigating the effect of various injuries on the growth of *Helianthus annuus*, showed that the removal of the cotyledons not only affected the total weight—as was to be expected, since a large portion of the plant's capital was thereby lost—but also markedly reduced the efficiency of the plant. Calculations from his weekly data of dry weights show that the efficiency index never rose above 14·24 per cent. per day as compared with the 18·46 per cent. of normal plants. It is not at all clear why the loss of the cotyledons should reduce the economy of working of the plant developed from the remaining portion, especially as the removal of *one* cotyledon and one of the first leaves had no such effect. It suggests that the cotyledons may contain a supply of some special material necessary for the proper development and efficiency of the plant; a possible analogy with the growth substances of animals occurs to one. The subject certainly requires further investigation.

Kiltz (*loc. cit.*) observed in *Nicotiana* a marked decrease in dry weight at the time when the seed was matured, reaching 2·98 per cent. in the case of *N. gigantea*. This might be explained by assimilation being brought to a standstill while respiration continued. The amount to be explained in this way is, however, very large, and Chodat, Monnier and Deleano[3] have described a restoration to the soil of mineral matter up to 40 per cent. of the dry weight of the mature plant. Whatever its cause, the phenomenon is of importance in all experiments where the dry weight of annual plants which have reached the fruiting stage is in question.

Summary.

Attention is drawn to the fact that the growth of an annual plant, at least in its early stages, follows approximately the 'compound interest law'. The dry weight attained by such a plant at the end of any period will depend on (1) the weight of the seed (or the seedling at its start),

[1] Experimental and Research Station, Cheshunt, Herts., Report III, p. 24, 1917.

[2] F. Gericke: Experimentelle Beiträge zur Wachstumsgeschichte von *Helianthus annuus*. Inaug. Diss., Halle, 1–43, 1909.

[3] Bull. de l'Herbier Boissier, vii. 350 and 948, 1907.

representing the initial capital with which the plant starts; (2) the average rate at which the plant makes use of the material already present to build up new material: this represents the rate of interest on the capital (material) employed; (3) the period of growth.

The plant is continually unfolding its leaves and increasing its assimilating power. Successive increases in the weight of the plant cannot therefore be treated as a discontinuous geometric series, as if the new material (interest) were added at the end of daily or weekly periods. New material is added continuously during daylight, and during rapid growth the plant is continuously, or nearly continuously, unfolding its leaves and increasing its assimilating rate. The growth of the plant more nearly approximates to money accumulating at compound interest where the interest is added continuously. The simple equation which best expresses the growth relations of active, annual plants is $W_1 = W_0 e^{rt}$, where W_1 = the final weight, W_0 = the initial weight, r = rate at which the material already present is used to produce new material, and t = time.

The term r is an important physiological constant, for it is a measure of the efficiency of the plant in the production of new material; the greater r is, the higher the return which the plant obtains for its outlay of material.

The rate of interest, r, may thus be termed the '*efficiency index*' of dry weight production, for not only is it a measure of the plant's efficiency but it is also an exponential term in the equation expressing the growth of the plant. In some forms of *Helianthus* the average efficiency index for the period up to the formation of the inflorescence may reach 0·1763 (i. e. 17·63 per cent.) per day.

It is suggested that in all experiments (such as water cultures, pot experiments) dealing with the production of vegetative material the efficiency index be calculated. The relative efficiency of different plants and of the same plant at different stages can thus be determined; also the effect on the efficiency index of various external conditions.

A small difference in the 'efficiency indices' of two plants (resulting, for example, from a slightly greater rate of assimilation or a more economical distribution of material between leaves and axis) may lead to a large difference in final weight. In oats, for example, an increase of 6 per cent. in assimilation might lead to an increase of 50 per cent. in dry weight at the end of 100 days.

The data of earlier workers show that the 'efficiency index' is highest in the early stages of growth, and then falls slightly. In *Helianthus*, *Cannabis*, and *Nicotiana* it falls sharply at the beginning of the reproductive period when the inflorescence first appears.

There is evidence that annual plants at the end of their period of growth may lose considerably in dry weight.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
LONDON.

The 'Brown Rot' Diseases of Fruit Trees, with Special Reference to Two Biologic Forms of Monilia cinerea, Bon. I.

BY

H. WORMALD, M.Sc. (Lond.), A.R.C.Sc.

Mycological Department, South-Eastern Agricultural College, Wye, Kent.

With Plates XXV and XXVI.

CONTENTS.

PAGE

I. INTRODUCTION 361
II. HISTORICAL 363
III. GENERAL OBSERVATIONS 367
(*a*) The Occurrence of *Monilia fructigena* and *M. cinerea* on *Prunus* spp. . . 367
(*b*) The 'Wither Tip' Disease of Plum Trees in 1918 368
(*c*) Sources of Infection on Plum Trees 369
(*d*) An Epidemic Outbreak of a 'Blossom Wilt' of Plum and Cherry Trees in 1918 369
(*e*) *M. cinerea* and *M. fructigena* on *Pyrus* spp. 370
IV. METHODS ADOPTED IN ISOLATING PURE STRAINS 371
V. INOCULATION EXPERIMENTS WITH *M. CINEREA* AND *M. FRUCTIGENA*:
(*a*) Apples inoculated with Strains of the Two Species 373
(*b*) Plums ,, ,, ,, ,, ,, ,, ... 381
(*c*) Plum Flowers inoculated with Strains of *M. cinerea* 387
(*d*) Apple ,, ,, ,, ,, ,, ,, ... 389
VI. CONCLUSIONS 397

I. INTRODUCTION.

IT has long been common knowledge among horticulturists and plant pathologists that the fruits of the cultivated species of the genera *Pyrus* and *Prunus* are frequently attacked by fungi which, gaining access to the nutritious parenchymatous cells of the fruit through wounds or abrasions of the skin, rapidly develop within the tissues. The progress of the disease is indicated by a discoloration of the affected cells, the resulting brown colour often showing a striking contrast with the healthy tissues, particularly in the case of immature fruit with pale green skins (see Fig. 4).

[Annals of Botany, Vol. XXXIII. No. CXXXI. July, 1919.]

The most important of the fungi producing these symptoms are to be referred to that genus of the 'Fungi Imperfecti' known as *Monilia*. Although these Monilias are able to continue from year to year without the interpolation of an ascigerous stage, such a stage has been observed, and it is now customary to allocate the species to the ascomycetous genus *Sclerotinia*.

The effect produced on the fruit by these species of *Monilia* is commonly known as 'Brown Rot' in this country, and this term is also now generally applied to the various diseases of fruit trees caused by these fungi. On the Continent the same diseases are referred to as 'Rot brun', 'Fäulniss der Früchte', 'Grind oder Schimmel des Obstes', 'Schwarzfäule', 'Blüten- und Zweigdürre', 'Muffa o marciume dei frutti', &c. The result of the development of the Monilias within the tissues is not usually, however, a 'rot' in the sense of putrefaction, since the organs attacked become so permeated with the mycelium that the whole forms a stromatic mass which generally tends to become more or less indurated rather than rotten. Thus the fruit, when so infected, instead of decaying and disintegrating, often becomes desiccated, and may remain on the tree in a 'mummified' condition for some months.

Towards the end of the nineteenth century serious losses to the cherry crops on the Continent, the flowers, fruit, and often branches being killed, led to an investigation of the cause of the outbreaks. The disease, at first attributed to the action of frost, was soon found to be due to epidemic attacks of a *Monilia*. It is now generally recognized that although the Brown Rot fungi are usually associated with fruit-rots they also cause blossom-wilts, destroy young twigs, and often kill branches by producing cankers which, girdling them and penetrating to the xylem elements, obstruct the course of the transpiration current and so cause the desiccation and death of those parts distal to the cankers.

The fruit is also liable to infection after it is picked, and apples in the store often suffer severely, for, since the disease under certain conditions is contagious, one affected apple may transmit the rot to those around it. Under storage conditions apples attacked by *M. fructigena* sometimes become quite black, a fact recorded in this country as early as 1885 by Worthington G. Smith.

That the Brown Rot fungi are recognized by mycologists and phytopathologists as of paramount importance is shown by the numerous papers and articles which have been published on the subject.[1] Many of these articles are merely records of the occurrence of the diseases, and relatively few supply scientific evidence for the conclusions arrived at. Of the experimental work, particularly that carried out on the Continent, very few investigators employed 'pure culture' methods in carrying out inoculations,

[1] See Bibliography on pp. 398–403.

and this, together with the fact that many workers still refuse to recognize that there are at least two distinct species of *Monilia* causing Brown Rot diseases, renders much of the work valueless as scientific records.

II. Historical.

In previous papers where the present writer (1917–18) described two specific diseases, on apple trees and plum trees respectively, literature was cited which related more particularly to the two diseases or to results obtained during the course of experiments connected with them, and no attempt was made to obtain a complete review of the papers dealing with those diseases of fruit trees caused by species of *Monilia*. Since the object of the present article is to show the relation which the two diseases bear to one another and to other Brown Rot diseases, a more general historical survey of our knowledge of the subject is desirable.

The earliest record of the occurrence of a *Monilia* on fruit appears to have been one by Persoon, who, finding a fungus producing tufts of moniliform chains of conidia on the decaying fruits of *Pyrus communis*, *Prunus domestica*, and *Amygdalus persica* in the year 1796, named it *Torula fructigena*. Five years later he changed the name to *Monilia fructigena*, and this is the name which is still usually applied to the conidial stage of the fungus. The former name was, however, frequently used for some time by certain workers, e.g. Albertini and Schweinitz (1805), Fuckel (1869), Saccardo (1873), Rabenhorst (1844–1853).

In 1817 Kunze and Schmidt named the fungus *Oidium fructigenum*, and this name was retained by Ehrenberg (1818), Fries (1829), Duby (1830), Cooke (1871), and Worthington Smith (1885). Other names by which it has been known are *Acrosporium fructigenum*, used in 1822 by Persoon himself, but not adopted by other botanists, *Oospora fructigena* and *O. candida*, as used by Wallroth (1833), and *Oidium Wallrothii*, by von Thümen (1875), who, however, in 1879 changed it to *O. fructigenum*.

Schröter, in 1893, from analogy with other species of *Monilia* producing an ascigerous stage, concluded that *M. fructigena* was the conidial stage of an ascomycete, and transferred it therefore to the genus *Sclerotinia*; in this connexion Prillieux (1897) wrote, 'ce n'est que par analogie que l'on peut regarder ce parasite comme une Pezize réduite à sa forme imparfaite de fructification'. In 1903 Ritzema Bos proposed that the fungus should be referred to the genus *Stromatinia*; this name has not been generally accepted, though adopted by Delacroix and Maublanc in their 'Maladies des Plantes cultivées'.[1]

The apothecial stage of *Monilia fructigena* was discovered on the Continent by Aderhold in 1904, thus confirming Schröter's conclusion regarding the ascomycetous nature of the fungus. Norton (1902) had two

[1] Loc. cit., vol. ii, p. 255. (Paris, 1909).

years previously found apothecia which had developed from mummied peaches; this form Norton himself referred to *Sclerotinia fructigena*, but Aderhold and Ruhland (1905) concluded that it was *S. cinerea*.

Westerdijk, in 1912, discovered a *Sclerotinia* on mummied cherries which, from the dimensions of its asci and ascospores, appeared to be different from *S. fructigena*, *S. laxa*, or *S. cinerea*; the direct connexion between the ascospores and a conidial stage was not traced however.

That *Monilia* was a genus which was to be considered as of economic importance was recognized by von Thümen in 1875, and in the following year by Hallier. Soon epidemic outbreaks of diseases caused by Monilias of fruit trees on the Continent resulted in the publication of many papers on the subject, particularly during the last decade of the nineteenth century, when articles appeared under such well-known names as Schröter (1893), Wortmann (1895), Wehmer (1895–6), Frank (1898), Frank and Krüger (1897–9), Aderhold (1897), Woronin (1897–9), Goethe (1898), Behrens (1898), Müller-Thurgau (1900), Sorauer (1899), and others,[1] the years 1897–1900 being prolific in references to epidemic outbreaks of *Monilia* diseases. In Germany the first serious complaint of such an epidemic appears to have been in 1894; thus Frank and Krüger write in 1899, 'Die erste Klage über das epidemische Auftreten der Monilia-Krankheit... wurde in Deutschland im Frühling 1894 laut, wo die landwirtschaftliche Hochschule in Berlin über das allgemeine Absterben der Kirschenblüten in der Ortschaft Blankenfelde bei Berlin um Rat befragt wurde'.

Since 1900, Aderhold, Müller-Thurgau, and Sorauer have continued their observations, and others have taken up the work or recorded the damage caused, e.g. Ritzema Bos (1903), Molz (1907), Köck (1910), Muth (1910), Ewert (1912), Voges (1912), Westerdijk (1912), Broz (1913), and Eriksson (1913).

In 1818 Ehrenberg discovered a form on apricots which he named *Oidium laxum*; as with *O. fructigenum*, this fungus has also been referred to other genera, its synonomy being as follows:

Oidium laxum, Ehrenb. Sylv. (1818), p. 10, 22.
Acrosporium laxum, Pers. Myc. I (1822), p. 25.
Oospora laxa, Wallr. Flor. Crypt. (1833), n. 1574.
Monilia laxa, Sacc. et Vogl. Sacc. Syll. Fung. IV (1866), 35.
Sclerotinia laxa (Ehrenb.), Aderh. et Ruhl. (1905).

Aderhold and Ruhland (1905) found the apothecial stage of this fungus in 1904 developing from mummied apricots which had been placed in soil in pots and kept under observation; they cultivated the ascospores on artificial media and reproduced the conidial stage. Since that date the name *Sclerotinia laxa* has been generally reserved for the form producing

[1] See Bibliography.

the brown rot of apricots, e.g. Faes (1914), Chifflot and Massonnat (1915), Peglion (1917).

Bonorden in 1851 introduced the name *Monilia cinerea* for a form which he found 'auf faulenden Früchten'; as in the case of *M. fructigena*, Schröter, in 1893, referred it to the ascomycetous genus *Sclerotinia*.

In spite of Woronin's well-known papers of 1898–9, in which he submits convincing proof that *Sclerotinia fructigena* and *S. cinerea* can be readily distinguished even in the *Monilia* stage, other investigators refuse to accept his conclusions, and consider that 'Brown Rot' diseases of our cultivated fruit trees are caused by one species only, i.e. *S. fructigena*. Thus Frank and Krüger (1899)[1] say, 'Auch der von Woronin festgehaltenen Unterscheidung von *Monilia fructigena* Pers. und *M. cinerea* Bon. können wir wegen der Übergänge ihrer Merkmale nicht beistimmen, ein Standpunkt, auf dem auch Behrens und Wehmer zu stehen scheinen'. Eriksson (1913) also writes,[2] 'Ich mache hier keinen Unterschied zwischen *Monilia cinerea* und *M. fructigena*, da eine Speciesbestimmung nur nach der Farbe der Conidienpolster immer misslich ist'.

Although 'Brown Rot' has caused serious damage to the fruit crops in our own country for many years, no scientific investigations of the outbreaks have, until quite recently, been attempted, and references to the diseases have been practically confined to notifications of their occurrence and to the recommendation of methods of control adopted abroad. No attempt was made to compare the forms occurring here with those investigated by Woronin and others on the Continent, and generally it has been assumed that all outbreaks of 'Brown Rot' were caused by *Monilia fructigena*.[3]

In 1864 an article appeared in the 'Gardeners' Chronicle' over the initials M. J. B., describing a serious outbreak of a rot of apricots, the disease being caused by 'a little greyish mould, *Oidium fructigenum*'; a similar disease of apples and pears was assumed to be caused by the same fungus. Worthington G. Smith writes, in 1885, that 'The fungus (referred to *Oidium fructigenum*) is often extremely destructive to stored Apples when kept in damp or unventilated store rooms; the same pest destroys Pears, Plums, and other fruits.

'The warts are generally grey in colour, varying, however, in tint from cream to fawn colour, or different shades of grey.' It would seem from this description of the pustules that both *Monilia fructigena* and *M. cinerea* had been observed but not recognized as different species. The same writer had also noticed that 'The fungus has, in some instances, the power of changing the skin of the Apple to a jet-black colour'.

[1] Loc. cit., p. 203. [2] Loc. cit., p. 65.

[3] An article in the Gardeners' Chronicle of December 30, 1915, refers to *M. cinerea* and *M. laxa*, but only in relation to work done on the Continent.

Massee again recognized one species only, referring to it as *Monilia fructigena* in his 'British Fungus-Flora' (1893), and in 'A Text-Book of Plant Diseases' (1903); later (1910) he describes it as *Sclerotinia fructigena*. Cooke (1906) also attributed attacks of Brown Rot on apples, pears, cherries, and apricots to one species, *Monilia fructigena*.

Spinks (1915–16) has recently investigated 'A Black Rot of Apples', particularly with regard to its occurrence on cider apples, and finds that it is caused by *Sclerotinia fructigena*, in the restricted use of this name as defined by Woronin; this disease is probably identical with the one observed by Worthington Smith (1885).

In 1907 Salmon recorded outbreaks of Brown Rot on acid cherries, and in 1910–14 called attention to a serious 'Brown Rot' disease which destroys the blossom of apple trees and may invade the branches and produce cankers. The present writer (1917) undertook the further investigation of epidemic attacks of this 'Blossom Wilt and Canker Disease' of apple trees, which was particularly prevalent on the variety Lord Derby in the Weald of Kent, and found that the fungus responsible was not *Monilia fructigena*, which had been assumed to be the species causing the disease, but a grey *Monilia* conforming to descriptions of *M. cinerea*, Bon. Later a 'Wither Tip of Plum Trees' (1918) was found to be caused by a fungus morphologically similar to that occurring on 'Brown Rot Cankers' of apple trees, but was different from that form biologically, in that it was unable to establish itself in the flowering spurs of the apple, and so was incapable of inducing the typical 'Blossom Wilt' condition or of forming cankers on apple trees.

Meanwhile phytopathologists in the United States had been attracted to the investigation of outbreaks of 'Brown Rot' in America, where fruit-growers were subjected to great losses annually, caused by a species of *Monilia*, peach trees proving particularly susceptible to the disease. Peck (1881) found that *Oidium fructigenum* attacked the fruit through wounds, for he was unable to produce infection by placing conidia on the uninjured skin. In 1886 Arthur recorded a blossom infection of cherries in New York as having occurred during the previous summer, and three years later Erwin F. Smith (1889) described a blossom blight of peaches as caused by *Monilia fructigena*, another paper on the same subject by the same author appearing in 1891. In that year Humphrey also wrote on 'The Brown Rot of Stone Fruits', and he too referred the fungus to *M. fructigena*, which he believed to have a conidial stage only. During the nine years following, further papers by Humphrey appeared, and other investigators took up the work, e.g. Chester (1892), Taft (1894), Bailey (1894), Goff (1897), Quaintance (1900), Waugh (1900).

In 1902 Norton made an important discovery when he found the ascigerous stage of the Brown Rot fungus occurring on peaches in Maryland;

the apothecia were developing from mummied fruit which had been lying on the ground for some time in an old peach orchard. Many bulletins on Brown Rot appeared during the succeeding ten years, dealing chiefly with the therapeutics of the disease, which was still generally attributed to *Monilia fructigena*. Then a series of papers appeared, written by Jehle (1913), Matheny (1913), Cooley (1914), Conel (1914), Valleau (1915), Brooks and Fisher (1916), and Bartram (1916), in which the writers conclude that the Brown Rot organism occurring in America is identical with *Monilia cinerea*, Bon., of Europe. 'Peach Canker,' attributed by Jehle to *Sclerotinia cinerea* (1913) or *S. fructigena* (1914), is found by McCubbin (1918), working in Ontario, to be due mainly to *Valsa leucostoma*, though the initial lesions in many instances are caused by the 'Brown Rot fungus'.

The Brown Rot diseases are also common in Australia,[1] and in New Zealand, as shown by Mansfield (1916), Cockayne (1918), and Cunningham (1918).

Articles recording experiments bearing on the physiological activities of fungi frequently give some attention to the Sclerotinias of fruit trees, e.g. Behrens (1898), Schellenberg (1908), Bruschi (1912), Cooley (1914), and Brooks and Cooley (1916).

III. General Observations.

In addition to observations already recorded in reference to a 'Blossom Wilt and Canker of Apple Trees' (1917), and a 'Wither Tip of Plum Trees' (1918), examination of diseased fruit trees in various districts of Kent, and of specimens received from other sources, has yielded further interesting information relative to the occurrence and mode of parasitism of *Monilia cinerea* and *M. fructigena*, and the following notes supplement those records of observations in the open which have already been published.

(*a*) *The Occurrence of Monilia fructigena and M. cinerea on Prunus spp.*

It has been generally assumed that *M. cinerea* is wholly, or almost, restricted to the stone-fruit trees (*Prunus* spp.), and *M. fructigena* to species of *Pyrus*. This assumption has arisen from the observations recorded by continental workers, and does not necessarily obtain in this country; in fact, *M. fructigena* is often found on cherries and plums, while a form of *M. cinerea* is responsible for a serious Blossom Wilt of apple trees. The records of the occurrence of *M. cinerea* on stone-fruit trees on the Continent have been chiefly in reference to the disease on the 'Sauerkirsche'. The acid cherry trees grown in England are also very susceptible to attack from *M. cinerea*, the blossom, fruit, and branches being killed; in no instance have I found *M. fructigena* on the acid cherry. In Kent the sweet cherries

[1] Vide McAlpine's 'Fungus Diseases of Stone Fruit in Australia', p. 53. Melbourne, 1902.

are cultivated on a much greater scale than the acid cherries, and on these *M. fructigena* is frequently found on the ripening fruit, while *M. cinerea* not only occurs on the fruit, but may sometimes cause a serious Blossom Wilt, as in the spring of 1918.

Both species are found on ripening and mature plums; sometimes *M. cinerea* is the predominant species present, on other occasions *M. fructigena*. In 1916 two growers, one in Sussex, the other in the Maidstone district of Kent, complained of serious losses by Brown Rot at the time of picking; samples sent to Wye College were found in each case to be affected by *M. fructigena* only. On the other hand, an orchard of Czar and Bush plums, near Maidstone, visited by the writer at the time the fruit was being gathered in 1917, was found to be infected by *M. cinerea* almost exclusively, a few examples only being found with *M. fructigena*. In another orchard, plums (varieties Monarch and Victoria) were attacked by the two species almost equally; in some instances the same cluster of plums bore both species, and occasionally the two were found on one and the same plum (see Figs. 1 and 2).

Both species occur on damsons, *M. cinerea*, as far as my own experience goes, being the more frequent. On peaches I have found *M. fructigena* on the ripening fruit and *M. cinerea* on dead twigs, but have not yet met with apricots affected with Brown Rot.[1]

(*b*) *The 'Wither Tip' Disease of Plum Trees in* 1918.

This disease, which was prevalent in Kent in 1916, was practically absent in the following year, but reappeared with its former intensity in 1918, when it was again (as in 1916) associated with an attack of aphides (*Aphis pruni*). Specimens of the 'Wither Tip' disease, received from Worcestershire and Norfolk, also bore traces of aphides on the leaves. The simultaneous occurrence on plum trees of the attack by the aphis and the outbreak of the *Monilia* disease of the vegetative shoots lends support to the theory that the insect renders the trees more susceptible to 'Wither Tip', either by aiding the dispersal of the conidia, by carrying them from one shoot to another, or by so injuring and weakening the leaves by punctures that the fungus is able to invade the tissues of the leaves and then the shoots; probably both these factors are operative during a severe infection.

Observations in 1916–17 proved that on diseased shoots infected in 1916 pustules of *Monilia cinerea* appeared during the following winter and spring. An examination of the same twigs (which had been labelled on the trees) in 1918 showed that pustules with viable conidia were again to be found on some of them, though many bore no fertile pustules at that time. Such twigs, therefore, may serve as sources of infection for two years.

[1] While this paper was in the press apricot twigs, bearing a fungus morphologically identical with *M. cinerea*, have been received at Wye from Norfolk.

(*c*) *Sources of Infection on Plum Trees.*

It is only during years of abnormal weather conditions, such as obtained in the spring of 1918, that heavy losses are produced on sweet cherries by the Brown Rot fungi. The plum tree, however, proves generally to be more susceptible, and each year extensive damage to the crop is reported. *Monilia fructigena* appears to be confined to the fruit, and persists from year to year, so far as is at present known, only on the mummied fruit, in this country. *M. cinerea*, on the other hand, not only attacks the fruit, but also the flowers, leaves, young shoots, and branches, thus not only directly reducing the cropping powers of the tree, but providing numerous sources of infection for the developing fruit.

From observations made at Wye it was found that at the time the fruit is about half grown, pustules of *M. cinerea* may be found on

(1) mummied fruit, the result of infection during the previous year;
(2) flowers killed in the spring of the current year;
(3) newly-attacked leaves on the vegetative shoots of the current year;
(4) vegetative shoots killed during the previous year;
(5) cankers formed the previous year on the branches.

The 'Wither Tip' condition may occur also on the 'suckers' at the base of the trees, and on those shoots growing out from the stumps of trees cut down; these serve as sources of infection which may easily escape observation.

(*d*) *An Epidemic Outbreak of a 'Blossom Wilt' of Plum and Cherry Trees in* 1918.

In 1918 the yields of the plum and cherry crops were far below the average. From investigations made in Kent it appeared that the failure was largely due to *Monilia cinerea* aided by weather conditions particularly favourable for the dissemination and development of the fungus during the period when the trees were in bloom, the atmosphere being almost continually saturated with moisture for about a week. The following factors contributed in inducing the epidemic outbreak of the disease:

(1) The low temperature retarded the development of the flowers, which on opening remained receptive and liable to infection for a relatively long period; this was partly owing to the fact that the cold wet weather decreased the activities of pollinating insects.

(2) The successive expanding of the flowers extended over a period long enough for those primarily infected to produce pustules of conidia during the time that later flowers were in progress of opening; these flowers which expanded towards the end of the flowering period were

therefore liable to infection from two sources, viz. mummied fruit and infected flowers.

(3) The pustules borne by the mummied fruit became extremely pulverulent with conidia (the saturated atmosphere being particularly favourable for their development) during the period when the flowers were open.

Another important factor was the great fertility of the trees during the previous season, in consequence of which many plums and cherries had remained unpicked and had become infected with *Monilia*, with the result that they persisted on the trees as 'mummies' and served as the primary source of infection during the spring of 1918.

(e) *Monilia cinerea and M. fructigena on Pyrus spp.*

The assumption that *Monilia cinerea* rarely occurs on the 'cored fruit' has been negatived during recent years, the fact that this species may frequently cause the destruction of 50 per cent. to 75 per cent. of the inflorescences of certain varieties of apple trees and that it is generally distributed throughout the fruit-growing counties in the south of England being sufficient evidence that on those varieties of apple trees it is far more dangerous than *M. fructigena*. The latter species occurs almost exclusively on the fruit, but occasionally extends from the infected apple into the fruiting spur (Fig. 3) or even into the branch to form a canker (Fig. 14). *M. cinerea*, on the other hand, infects the flowers and, rapidly advancing into the spurs, kills them, and cankers, girdling the branches, are often produced (see Figs. 10–13). The statement made in 1917 that I had never found *M. cinerea* on maturing or ripe apples still holds good.

With reference to the Brown Rot Canker disease, earlier observations appeared to show that the fungus produces fructifications on spurs and cankers during the winter and spring following their infection, and that subsequently the fungus produces no more pustules, but dies away, and the cankers become covered by the development of callus (see Fig. 16). More recent observations have shown that, although this is true as a general rule, in some instances infected spurs have developed pustules during the second year after infection. Thus in the summer of 1917 it was found that a few of the spurs labelled as being infected in 1915, and which had produced pustules in 1916, also bore pustules in 1917; in one instance a spur which had been infected from artificial inoculation of the inflorescence from a pure culture in 1916, and had borne fructifications in 1917, produced still other pustules with viable conidia in 1918, though the canker which had been formed at the base of the spur was by this time almost healed over by callus (see Fig. 13, which shows the spur and canker here referred to, photographed in March, 1918). In every case observed where a canker has been produced that has not completely girdled the

branch the lesion tends to become healed quite early (Fig. 16), and no more pustules develop on the cankered surface of the branch itself subsequent to the year following infection. When, however, the canker girdles and kills the branch the lesion cannot be healed and the fungus may persist in the tissues of the canker for several years. In the spring of 1917 large cankers bearing numerous pustules were removed from affected trees; some of these were placed in a pot on the ground, others were suspended at about 3 feet above the ground level. In January, 1919, those cankers which had been suspended still bore numerous powdery pustules (see Fig. 12); of those on the ground most had lost their bark and no pustules were present, while the rest bore a few pustules at the upper end. Such cankers, therefore, may serve as sources of infection for at least three years.

Another feature, not previously recorded, in connexion with this disease is the fact that when a leading branch is girdled by a canker, which, as already shown,[1] occurs about the end of June when the tree is growing vigorously, the upward flow of sap is suddenly checked and diverted into the buds immediately below the canker, with the result that these buds are stimulated to grow out into weak vegetative shoots instead of developing into fruiting spurs (Fig. 15). A similar result has been described and figured as occurring on plum trees affected with the 'Wither Tip' disease.[2] On pear trees I have met with *Monilia cinerea* on one occasion only, when it was found on a young pear about 1 cm. in length, while *M. fructigena* often occurs, causing a brown rot, on the ripening fruit.

Pyrus japonica is subject to a Blossom Wilt similar in general appearance to that of the apple tree. I have isolated the fungus in one case, and it proved to be similar physiologically and biologically to the form of *M. cinerea* obtained from plums and cherries.

IV. Methods adopted in isolating Pure Strains.

The strains of *Monilia* used in the experiments recorded in this paper were all 'pure', each being derived from a single conidium; the conidia were obtained for this purpose either directly, from naturally infected specimens bearing fructifications, or indirectly from cultures prepared with the object of inducing the development of conidia in those cases where mycelium only was present in infected material.

A method of isolating pure strains of *Monilia* from infected tissues containing mycelium has been described in previous papers (1917–18), and brief references have there been made to a method of obtaining strains from single conidia. The latter given in detail is as follows: Two watch-

[1] Vide Ann. Appl. Biol., vol. iii, p. 167.
[2] Vide ibid., vol. v, Plate VIII, Fig. 3.

glasses, held in forceps, are passed several times through a Bunsen flame; one is then inverted over the other and both allowed to cool, when into the lower is poured about 1 c.c. of sterilized distilled water. Particles of pulverulent pustules are brought in contact with the water, and the conidia, becoming detached, float on the surface. By means of a flamed platinum wire loop (1 mm. diam.) drops are transferred from the watch-glass to the surface of sterilized nutrient agar in a Petri dish. As this operation is carried out in a room set apart for culture work it was found possible to examine these drops directly, the cover of the dish being removed, under a low power of the microscope with but little risk of contamination. Since the conidia float on water the surface of the drop is brought into focus and examined for the presence of floating conidia. Generally with one loopful three drops of water can be deposited on the agar without recharging the loop, and this is a convenient number to examine before evaporation causes their obliteration. When a drop is found to contain a single conidium the cover of the dish is replaced and a ring of ink is made on the bottom of the dish to mark its position. Several such marked drops having been obtained, the dish is placed in a drawer at room temperature until the following day. After twenty-four hours the inverted plate is examined under the microscope with a 1-inch objective. One of the rings of ink is brought into the field and the microscope tube is gradually lowered until the agar is in focus as indicated by the appearance of numerous small granules; the layer of granules last seen before they disappear from view indicates the surface of the agar, and this is then examined at that level for the germinating conidium within the ring of ink, the blurred outline of which is still discernible.

It will, in general, be seen at this stage whether the conidium is truly isolated or whether contamination has taken place. In most instances the length of the germ tube and its mode of growth were also noted, and it was generally found that the germ tube of *M. fructigena* could be distinguished from that of *M. cinerea*.[1]

On the second day after isolation they are again examined and two or three of those which are found to be uncontaminated, and quite isolated from other germinating conidia, are transferred to another agar plate. Usually this transference is made after forty-eight hours, but occasionally, when the temperature of the room was low and growth had not been vigorous, the sporelings were removed on the third day after the isolation of the conidia. When the resulting mycelial discs are 2–3 cm. in diameter (representing about eight days' growth) small portions of the mycelium are removed from the periphery of the disc and placed on agar slants in test-tubes for future use, and when conidia are required sub-inoculations are also made on sterilized potato.

[1] Further details will be given in Part II of this paper.

In this way purity of culture is ensured, for should contamination arise from fungus spores, or by bacteria capable of growing on the culture medium employed, it would be detected during the forty-eight hours succeeding isolation, and should the sporeling be contaminated by bacteria which do not develop under those conditions and would therefore escape detection, they are eliminated by taking mycelium from the peripheral region of the mycelial disc after growth has proceeded for a few days. This method was the one adopted for obtaining the 'single spore strains' of *Monilia fructigena* and *M. cinerea* (used in the experiments described in this paper) when viable conidia were available, i. e. when the fungi were developing pulverulent pustules.

When the parasites occur as barren mycelium in the tissues of the host they are induced to produce conidia on sterilized potato as already described in the papers on 'A Blossom Wilt and Canker of Apple Trees'[1] (1917) and 'A Wither Tip of Plum Trees'[2] (1918). The conidia are then isolated and single spore strains obtained as described above.

Single spore strains of *Monilia fructigena* were prepared directly from the conidia of fertile pustules on apples or apple spurs, or indirectly from barren pustules (winter condition) by placing particles of the pustules on the nutrient agar, thus inducing vegetative growth for sub-inoculation on potato for conidia production. 'Black Rot' strains of *M. fructigena* were also obtained indirectly by first stimulating vegetative development from particles of the diseased apple flesh (permeated with mycelium) placed on the agar.[3]

V. Inoculation Experiments with *Monilia cinerea* and *M. fructigena*.

(*a*) *Apples inoculated with Strains of the Two Species.*

Experiments carried out on apples in 1916 with the object of comparing the action of that form of *Monilia cinerea* which causes the Blossom Wilt disease of apple trees with that of a strain of *M. cinerea* isolated from a plum affected with Brown Rot, and with that of a strain of *M. fructigena* also obtained from a plum, have already been described in a previous paper (1917). It was there shown that the mode of development of the Blossom Wilt form of *Monilia* under those conditions (growing on immature apples in the laboratory) was different from that of either of the other two forms in that the brown coloration of the affected areas rapidly became dark brown and finally black in the case of the Blossom Wilt strain, while the

[1] Loc. cit., p. 174. [2] Loc. cit., p. 33.

[3] The writer has found the method here described (slightly modified in the case of spores or conidia which do not float in water) of obtaining 'single spore strains' suitable for other fungi with reproductive bodies large enough to be easily seen under a low power of the microscope.

other two strains failed to induce this nigrescence except on fully matured apples inoculated some time after they had been picked and stored; it was also to be distinguished from the fact that on the apples so infected it produced very few pustules, or in some instances none at all, while the strains of *M. cinerea* and *M. fructigena* from plums readily developed pustules.

During 1917 another series of experiments was carried out using the same strain of the Blossom Wilt fungus (which, however, had been re-isolated from an apple spur infected from a pure culture) and other strains of *Monilia cinerea* and *M. fructigena*. In this series, Experiments Nos. 4 and 5 were made on apples growing on a tree in the open, Nos. 1, 2, 3, 6, and 7 on apples which were inoculated and kept under laboratory conditions. In the latter case, the fruit after inoculation was placed in a glass vessel which had been sterilized on the inside by a thorough cleansing with cotton-wool soaked in 95 per cent. alcohol. The actual inoculation consisted in making a small V-shaped cut through the skin, removing a particle of the tissues underneath, and inserting mycelium from an agar culture of the fungus, the skin being then turned back into position. When the resulting 'Brown Rot' had developed for about seven days, by which time the fungi had become well established in the tissues, the apples were placed under bell-jars on a slate slab (which had also been cleansed with alcohol), the jars being raised above the surface of the slab on glass supports 1 cm. in height, thus allowing for a circulation of the air. This method, though not excluding the possibility of infection by other organisms during the latter stages of the experiment, provided conditions less abnormal than would have been the case had the apples been kept in an enclosed space throughout the experiment, and as no other organisms appeared it is to be concluded that the results obtained were produced by the fungi inserted in the wounds.

In 1917 inoculation experiments on apple flowers showed that the strain of *Monilia cinerea* obtained from a 'withered tip' of a plum tree was unable to induce the Blossom Wilt condition on the flowering spurs of the apple; other experiments carried out in 1918 (described in detail in the present paper) with other strains from plum and cherry also failed to produce the Blossom Wilt on apple trees. The 'Blossom Wilt and Canker' form of *Monilia cinerea* is therefore distinct biologically from that occurring generally on the species of *Prunus*, and it is proposed, for convenience, to refer to it in describing the following experiments as *Monilia cinerea* forma *mali* and the form on plum and cherry as *M. cinerea* forma *pruni*. Strains of *Monilia* obtained from America have also been used, and, as these have certain characters in common which distinguish them from *M. cinerea* as found in Europe,[1] they will be referred to as *M. cinerea* forma *americana*.

[1] Vide Ann. Appl. Biol., vol. iii, No. 4, p. 180.

EXPERIMENT 1.

Strains used { *Monilia cinerea* forma *mali*.
M. fructigena, strain from apple spur.

Two apples (var. Bramley's Seedling) were each inoculated on one side with a strain of the apple Blossom Wilt form of *Monilia cinerea*, and on the opposite side with a strain of *M. fructigena* obtained from an apple spur which had become infected through the fruit. The inoculations were made on September 14, 1917, with the following results:

September 21. In both apples on the side inoculated with *Monilia cinerea* the rot had extended 2·5–4·5 cm. from the point of inoculation; no pustules were present, but conidiophores had developed along the cut at the place where the inoculation was made; the discoloration was a deep brown, with darker, more or less concentric zones; many of the lenticels were marked with dark circles.

On the side inoculated with *M. fructigena* the rot had extended 3–3·5 cm.; a few yellow pustules, to 2 mm. in diameter, were present, and conidiophores had also developed along the wound; the discoloration of the surface was a bright brown, except in the immediate neighbourhood of the point of inoculation, where the colour was darker for about 1 cm.

October 12. *M. cinerea* had extended through about two-thirds of each apple, the surface on that side being *black* in colour; the skin was hardly shrunken and bore no pustules. *M. fructigena* had made less progress, about one-third of the surface being affected; the skin was bright *brown* in colour, much shrunken, and bore numerous yellow pustules.

October 25. There was no further change except that the shrinkage was more pronounced, particularly on the side infected with *M. fructigena*.

These results were essentially the same as those obtained in 1916, when a strain of *M. fructigena* obtained from a plum had been compared with the Blossom Wilt form of *M. cinerea* (see Figs. 4 and 5).

EXPERIMENT 2.

Strains used { *Monilia cinerea* forma *mali*.
Monilia cinerea forma *pruni*.

Two apples were each inoculated on one side with the apple Blossom Wilt strain used in Experiment 1, and on the other side with a 'Wither Tip' strain of *M. cinerea* from a plum tree. The inoculations were made on September 14, 1917.

RESULT.

	Sept. 21.	*Oct.* 12.
Apple No. 1 on side inoculated with *M. cinerea* forma *mali*.	Discoloration extending 4–4·5 cm. from inoculated wound, dark brown in zones, no pustules present, conidiophores along cut	A little more than half the surface of the apple *black*, not shrunken; no pustules, but a few small barren papillae present.
Apple No. 1 on side inoculated with *M. cinerea* forma *pruni*.	Discoloration extending 2·5–3 cm., from wound, bright brown with a slightly darker margin; a few minute scattered pustules.	This side (rather less than half surface) *brown* and distinctly marked off from the black portion; not shrunken; no powdery pustules, but numerous papillae present.
Apple No. 2 on side inoculated with *M. cinerea* forma *mali*.	Discoloration extending 4–6 cm. dark brown in zones; a few small scattered papillae; conidiophores along cut.	As in Apple No. 1.
Apple No. 2 on side inoculated with *M. cinerea* forma *pruni*.	Discoloration extending 1·5–3 cm. from wound; many small pustules present and conidiophores along the cut.	Numerous small grey pulverulent pustules present; skin distinctly shrunken and wrinkled, *brown*.

October 25. No further definite change in any of the apples except that the shrinkage is a little more pronounced.

The two strains used in this experiment were the same as those used in the inoculation of apple flowers as described in the paper on 'A Wither Tip of Plum Trees' (1918); in that paper it was shown that the Wither Tip strain failed to induce the typical 'Blossom Wilt' condition on an apple tree, while on the same tree inflorescences inoculated with the Blossom Wilt strain quickly succumbed. In the case of these two strains the degree of the virulence of their parasitism on apple flowers is associated with differences in their physiological relations with the host tissues when growing on the fruit of the apple tree.

EXPERIMENT 3.

Strains used:
- *Monilia cinerea* forma *mali*.
- *Monilia cinerea* forma *pruni*.
- *Monilia cinerea* forma *americana*.
- *Monilia fructigena*, strain from an apple spur.
- *Monilia fructigena*, strain from a 'Black Apple'.

Fifteen apples (all of one variety, viz. Bramley's Seedling) were selected as being free from blemishes, divided into groups of three, and the groups respectively inoculated with the five strains of *Monilia*. The strains of the forms *mali* and *pruni* of *M. cinerea* were the same as those used in Experiment 2, and the strain of *M. fructigena* obtained from an apple spur was that used in Experiment 1. The present experiment, therefore, served as a duplicate of the previous ones, and also introduced for com-

parison the American form of *M. cinerea* and a 'Black Apple' strain of *M. fructigena*.

The apples were inoculated on October 26. 'Brown Rot' soon appeared in all of them except one of those inoculated with the 'Black Apple' strain of *M. fructigena*, and as, with this exception (in which infection failed), the results obtained in any one series were approximately uniform, they are described in the table in general terms.

RESULT.

Strain of Monilia.	*Nov.* 3.	*Nov.* 30.
M. cinerea f. *mali.*	The rot had extended 2·8–4·0 cm. from point of inoculation; pustules absent; distinct but irregular dark brown zone at 1·5–2·5 cm. from the wound.	One apple quite black, the other two black over nearly the whole surface, the remaining portion being dark brown; pustules minute, few in number; skin very slightly wrinkled.
M. cinerea f. *pruni.*	Extension of rot 2·2–3·5 cm.; numerous grey pustules on one apple and a few on the others.	Surface of all three a bright brown; no nigrescence except for a few millimetres immediately round the wound; numerous grey pustules about 1 mm. diam.; skin much shrunken and wrinkled, particularly where the pustules were closely aggregated.
M. cinerea f. *americana.*	Extension of rot 2·0–3·0 cm.; discoloration brown, with a darker zone at 0·5–1·0 cm. from wound; pustules few, small (about 1 mm. diam.), and grey.	All three quite black with the exception of a small brown patch on one of them; pustules few, small, grey; skin slightly wrinkled.
M. fructigena. Strain from apple spur.	Extension of rot 1·8–2·5 cm.; pustules absent; discoloration brown, with a narrow peripheral zone a little darker.	All three brown to dark brown; pustules yellow, to 3 mm. diam., very numerous; surface of apples much shrunken and wrinkled.
M. fructigena. Strain from a 'Black Apple'.	Extension of rot 1·5–2·7 cm.; pustules absent; discoloration brown, with a narrow peripheral zone a little darker.	All three brown to dark brown; pustules yellow, to 3 mm. diam., very numerous; surface of apples much shrunken and wrinkled.

EXPERIMENT 4.

Strains used { *Monilia cinerea* forma *mali.*
Monilia fructigena, strain from an apple spur.

On July 6, 1917, six apples (var. James Grieve), growing in the open, were inoculated with *M. cinerea* and eight with *M. fructigena*; a number of apples wounded but not inoculated served as controls. In each series infection failed in two of the apples. Those which became infected had all fallen by July 20, so that it was decided to repeat the experiment, and other apples were inoculated with the same strains on July 24, as described under Experiment 5. The results obtained to July 20 are to be noted, however, for comparison with those recorded in the next experiment.

The apples infected with *M. fructigena* became within eight days discoloured over more than half the surface; in one case the whole surface was brown. At this stage a pale raised circular pustular zone, covered by the epidermis, had developed at approximately 1 cm. from the point of inoculation; a few days afterwards yellow pulverulent fructifications of *M. fructigena* burst through the skin in that zone, and later numerous pustules appeared on the rest of the surface.

Of the apples infected with *M. cinerea* two had become discoloured over three-fourths of the surface, the other two over the whole surface, but *no pustules* had developed.

In both series the apples in which inoculation had failed to produce infection were still on the tree on July 20, as were also all the control apples.

Experiment 5.

Strains used { *Monilia cinerea* forma *mali.*
Monilia fructigena, strain from an apple spur.

This experiment was carried out on apples (var. James Grieve) growing in the College plantation. The apples were inoculated on July 24 by making in each a single puncture with a sterile needle, and inserting in the puncture a particle of a pustule obtained from a pure culture of the fungus growing on sterilized potato. Ten apples were inoculated with each of the two strains, and, to serve as controls, ten apples were punctured but not inoculated.

Result.

	July 26.	*July* 30.
M. cinerea f. *mali.*	Rot had commenced in all the apples, the brown areas round the punctures varying from 3 to 13 mm. in diameter.	About half the surface of each was discoloured; four of the apples bore no pustules whatever, four had a few scattered pustules, and on one only were they at all comparable in number to those occurring on the apples infected with *M. fructigena*; the pustules were ashy grey in colour and the largest was about 0·5 mm. in diameter.
M. fructigena.	Rot had commenced in all, the affected area being 3–13 mm. in diameter.	About half the surface of each was discoloured and all bore yellow (light buff) pustules to 2 mm. in diameter; the pustules were almost localized to a definite circular zone at about 1 cm. from the point of inoculation.

No rot appeared on any of the control apples.

Strong winds, which occurred early in August, detached all the inoculated apples except one. The exception was one infected with *M. fructigena*, which had been in contact with the bark of the branch; the development of numerous pustules served to attach the apple to the

bark, so that although the stalk had become almost severed at the point of its insertion on the fruiting axis, being connected only with a narrow strand of fibrous tissue, the apple still remained *in situ.*

Of the apples found on the ground those inoculated with *M. fructigena* were by this time almost entirely covered with yellow pulverulent pustules, while on those inoculated with *M. cinerea* the pustules were few and small or absent altogether. Most of the apples used as controls were still on the tree.

Experiment 6.

Monilia fructigena, when growing in stored apples, frequently causes a 'Black Rot' similar in appearance to that produced on those apples artificially inoculated with *M. cinerea* f. *mali*. Spinks (1915), investigating a 'Black Rot' of cider apples, found that 'the apples of the same variety showed the same type of rot irrespective of the fungus with which they were infected', and that 'it seemed certain that the type of rot produced in an apple by *Monilia fructigena* depended only on the apple itself'. An experiment carried out at Wye in 1916 shows that this is not invariably the case, as seen by the results here recorded.

Strains used { *Monilia fructigena*, strain from a plum.
Monilia fructigena, strain from a 'Black Apple'.

Four apples (variety Bramley's Seedling) were each inoculated on one side with a strain isolated from a pustule of *M. fructigena* growing on a plum, and on the other side with a strain isolated from the fleshy tissue of an apple affected with 'Black Rot'; the inoculations were made on September 8.

Result.

	Side inoculated with Plum strain.	*Side inoculated with 'Black Apple' strain.*
September 13.	A brown discoloration extended from point of inoculation for 2·5–3 cm.; pustules from 60 to 90 in number. The discoloration was a uniform brown, no dark circles at the lenticels.	Discoloration extended 2·5–3 cm. from point of inoculation; the pustules on the four apples numbered respectively 1, 4, 2, and 13. The discoloration was darkest towards centre of affected area, shading off to a brown of the same tint as that on side infected with the plum strain; dark brown circles round lenticels.
September 18.	The whole side was now a uniform brown; no black circles at lenticels; pustules numerous.	Brown colour still darker round point of inoculation; pustules few and scattered; black circles round the lenticels.
October 12.	Pustules numerous, in an almost continuous layer, but showing some tendency to form concentric circles round the centre of infection; the skin, where it could be seen between the pustules, was dark brown to black.	Pustules few and scattered; skin dark brown to black, nigrescence most conspicuous immediately round the lenticels.

Since this experiment pointed to a difference in the physiological reaction of the two strains to the tissues of the apple in which they were growing, the experiment was repeated in 1917 (see Experiment 7) for confirmation of the results, using the same two strains, which in the interval had been cultivated on sterilized media in the laboratory. The results were even more striking than in 1916, showing that the difference was still maintained even after cultivation for twelve months, under similar conditions, as saprophytes.

Experiment 7.

Strains used—as in Experiment 6.

Two apples were inoculated, as in the previous experiment, on one side with the strain from a plum, and on the other with the 'Black Apple' strain; the inoculations were made on September 14, 1917.

Result.

	Side inoculated with Plum strain.	*Side inoculated with 'Black Apple' strain.*
September 21.	The affected area extended 4–5·5 cm. from point of inoculation; it was bright brown in colour with a rather darker narrow peripheral zone. Numerous yellow pustules, to 2 mm. diam., were present, growing in irregular concentric circles, the first at 1 cm. from the centre.	The rot extended 1–2·3 cm. from the point of inoculation; one apple was of a distinctly darker brown on this side than on the side infected with the plum strain, particularly in a zone 1–2 cm. from the centre, while on the second apple this deeper shade was represented only by two blackish spots at 1 cm. from the centre. No pustules were present, but conidiophores had developed along the wound.
October 12.	The whole of this side was now a bright brown and bore numerous yellow pustules; it was much shrunken and the skin wrinkled.	Skin black and but very slightly wrinkled; no pustules present.

On October 12 the line along which the two strains met was sharply marked, and the black colour of the one side, with its almost smooth skin and absence of pustules, formed a striking contrast to the other (Fig. 7).

By October 25 there was no further change except that the shrinkage was more pronounced, particularly on the side infected with the plum strain.

Summary of Results of Inoculations on Apples.

Monilia fructigena and *M. cinerea*, when growing on apples, can be distinguished morphologically, the former producing large (up to 2 or even 3 mm. in diameter) yellow pustules, the latter smaller grey ones.

In each species there are two forms which are different physiologically: one which, when growing under the conditions which obtained during the course of the experiments, develops many pustules, and produces a brown

discoloration throughout the affected tissues, and another which develops few or no pustules and causes a discoloration which, primarily brown, gradually becomes darker in the superficial layers until eventually the whole surface is quite black.

Growing apples infected with either species are far more easily detached from the tree than the sound fruit, unless they become attached to the bark, or to other apples in contact with them, by means of the pustules at the point of contact. This fact suggests a reason for the rare occurrence of *M. cinerea* on apples, for the small and comparatively few pustules which it produces appear to be insufficient to attach the fruit to the tree even should infection occur. The writer has occasionally found *M. cinerea* f. *mali* on the very young fruit, but never on any approaching maturity, even on trees severely attacked by the Blossom Wilt disease, while *M. fructigena*, which usually develops numerous large pustules on maturing apples, is extremely common.

Apples, when infected with a form which produces numerous pustules, soon become shrunken and wrinkled, while the skin of those infected with strains which do not develop pustules under those conditions often remains firm and smooth for some time after it has become quite black. The rapid desiccation of the tissues when pustules are present is probably due to two causes: (*a*) the rupture of the epidermis permitting evaporation from within; (*b*) loss of water by reason of the respiratory and transpiratory processes in the cells of the hyphae and conidiophores of the pustules.[1]

(*b*) *Plums inoculated with the Two Species.*

These experiments were carried out in two series: (1) inoculations made with the object of comparing the morphology of the two species when growing on plums; (2) inoculations with strains of *Monilia cinerea* for comparing results obtained with the form *mali* with those of the form *pruni*.

The results obtained from inoculating growing plums with a 'Wither Tip' strain of *M. cinerea* have already been published (1918). A similar experiment was made about the same time, i.e. July 1917, using *M. fructigena*; the resulting brown rot was indistinguishable in general appearance from that produced by *M. cinerea*, but the pustules which developed were larger, and yellow in colour. Both species were able to cause infection of uninjured plums in contact with diseased ones by the development of modified pustules at the point of contact, a pad of hyphae being formed which was able to penetrate the skin.

For confirmation of these results, and to obtain a more exact comparison of the two when growing on plums, an experiment was started

[1] When apples bearing numerous *Monilia* pustules are placed in a closed glass vessel, moisture condenses on the internal surface of the vessel within a few hours.

in which inoculations were made simultaneously with the two species on plums in close proximity and also on opposite sides of the same plum.

Experiment 1.

Strains used { *Monilia fructigena*, strain isolated from a plum. *Monilia cinerea*, strain isolated from a 'Wither Tip' twig of a plum tree.

Plums (variety Victoria) growing in the open were numbered and labelled as follows:

1, 2, and 3 were each a pair of plums at the same node; one of each pair was inoculated with *M. fructigena*, the other with *M. cinerea*. 4, 5, and 6 were single plums, each inoculated on one side with *M. fructigena*, and on the other side with *M. cinerea*. In the accompanying table the plum or side inoculated with the former is indicated by *f*, and with the latter by *c*.

The inoculations were made on July 17, 1917, by making, in each case, a single puncture through the skin with a sterile needle and placing conidia from a pure culture in the wound.

Six plums, three inoculated with M. fructigena, three with M. cinerea.	*No. of mm. through which rot had extended from point of inoculation by July 20.*	*Result on July 26.*
1 *f*	5–7	The whole surface was discoloured; yellow pustules were present; the fruit-stalk was brown for 3 mm.
1 *c*	5–7	,, numerous grey pustules ,, ,, 4 mm.
2 *f*	8–9	,, yellow pustules ,, ,, 3 mm.
2 *c*	6–8	,, grey ,, ,, ,, 12 mm.
3 *f*	5–8	,, yellow ,, ,, ,, 3 mm.
3 *c*	4–6	,, grey ,, ,, ,, 3 mm.
Three plums, each inoculated on one side with M. fructigena, on the other with M. cinerea.		
4 *f*	9–12	The two fungi had met in a plane approximately midway between the two points of inoculation; *M. fructigena* had produced fairly numerous yellow pustules, *M. cinerea* numerous grey ones; the stalk was brown for 8 mm.
c	6–9	
5 *f*	8–9	As in No. 4, but stalk brown for 3 mm.
c	5–7	
6 *f*	9–10	As in No. 4, but stalk brown for 12 mm.
c	5–6	

The discoloration of the plums was a dull purple brown with both species; on the whole, the distance from the point of inoculation reached by *M. fructigena* after three days was a little greater than that attained

by *M. cinerea*, the maximum extension for the former being 12 mm. and for the latter 9 mm. The brown colour on the fruit-stalk extended upwards from the point of insertion on the plum, indicating that the disease was advancing from the fruit, along the stalk, towards the branch.

The pustules of *M. fructigena* were to 1·5 mm. in diameter, usually about 1 mm., and their colour was 'Light Buff' of Ridgway's colour charts;[1] those of *M. cinerea* were conspicuously smaller, reaching a maximum diameter of 0·8 mm., the usual diameter being about 0·4 mm., and their colour was 'Smoke Grey' to 'Light Greyish Olive'.

On July 27 plums Nos. 2*f* and 2*c* were removed from the tree and photographed, together with a sound plum from the same tree (Fig. 8); the plum No. 6 was also photographed on the same day (Fig. 9).

The results obtained in this experiment prove conclusively that *M. fructigena* and *M. cinerea* are equally able to cause a 'Brown Rot' of plums, and that under these conditions the two species are morphologically quite distinct, as shown by their fructifications.

Experiment 2.

The experiment recorded here is one of a series carried out with the object of ascertaining whether the two forms *mali* and *pruni* of *Monilia cinerea* could be distinguished when growing on plums. The experiments were suggested by the fact that of all the strains of *M. cinerea* obtained from plums or cherries (more than 20 in all), not one could be identified, by the characters given later in this paper,[2] as forma *mali*, and a reason was sought for this absence of the apple form from species of *Prunus*. *M. cinerea* f. *mali* produces pustules less freely on sterilized potato and on apples (as shown in preceding pages) than f. *pruni*, and it was thought that the former, if it did occur occasionally on plums, was unable to become established there, for experiments on apples in the open showed that pustules were necessary for the diseased fruit to be retained on the trees as mummies. Preliminary experiments in the laboratory proved that the form *mali* grows readily when inoculated into picked plums, again with a tendency to develop fewer pustules than does the form *pruni*.

During the summer of 1918 plums growing in the College plantation were inoculated on June 21, June 24, and July 15; on each occasion the strains used were:

M. cinerea forma *mali*, strain from a 'Brown Rot Canker'.
" " *mali*, " " an apple spur.
" " *pruni*, " " a mummied plum.
" " *pruni*, " " a mummied cherry.

[1] Color Standards and Nomenclature. Washington, 1912.
[2] These features will be discussed in Part II of this paper.

All the strains readily produced on the infected plums the characteristic 'Brown Rot' and numerous pustules. A slight difference could be detected in the productivity of pustules in the two forms in a similar experiment started a little later in the season, viz. Aug. 1, when the fruit was approaching maturity, and the detailed results of this experiment are here tabulated. The pustules are described as 'numerous', 'many', or 'few' in the following sense:

Pustules numerous—occurring scattered over the whole surface and often also becoming confluent to form continuous zones or irregular patches.

Pustules many—scattered over the whole affected surface; continuous patches absent or few and small.

Pustules few—isolated and scattered over a portion (half or less) of the discoloured area.

Two trees (variety Victoria) were reserved for the experiment, ten plums being inoculated on each tree as shown in the table.

The method of inoculation adopted was similar to that used in Expt. 1.

	Strains used in the inoculations (Aug. 1).	*Plum No.*	*Aug. 6.*	*Aug. 13.*	*Aug. 28.*
TREE I.	*M. cinerea* f. *mali.* Strain from a 'Brown Rot' Canker on an apple tree.	1	Half the surface affected; pustules few.	Whole surface discoloured and wrinkled, plum shrunken; pustules many; two other plums in contact with it were discoloured over their whole surface and bore a few pustules.	The three plums were shrunken and wrinkled and bore many pustules.
		2	Half the surface affected; pustules absent.	Whole surface wrinkled, plum shrunken; pustules many; in contact with inoculated plum were one wholly discoloured and another with $\frac{1}{3}$ of the surface affected, both with a few pustules.	The plum primarily inoculated had infected 4 others; all were shrunken and bore many pustules.
		3	The rot had extended 1·5–2 cm. from point of inoculation; pustules none.	Whole surface affected; pustules many; another plum, infected from contagion, showed a rot extending 1–1·5 cm. from the point of contact.	Both were shrunken and bore many pustules.
		4	The rot had extended 1·3–1·8 cm.; pustules none.	Whole surface affected; pustules many.	Fallen.
		5	Half the surface affected; pustules none.	Whole surface affected; pustules few.	Much shrunken and bearing many pustules.

	Strains used in the inoculations (*Aug.* 1).	*Plum No.*	*Aug.* 6.	*Aug.* 13.	*Aug.* 28.
TREE I.	*M. cinerea* f. *pruni*. Strain from a mummied plum.	1	Nearly half the surface affected; pustules none.	Whole surface affected; pustules numerous; in contact with it were one wholly discoloured with two almost continuous zones of confluent pustules, and another with half the surface affected and bearing few pustules.	One other had also become infected from contagion; all four were shrunken and bore numerous very pulverulent pustules becoming confluent and forming patches to 5 mm. diam.
		2	Ditto	Whole surface affected; plum shrunken and wrinkled; pustules numerous.	Pustules more numerous; plum still more shrunken and wrinkled.
		3	One-third of the surface affected; pustules none.	Ditto	Pustules more numerous.
		4	Ditto	Ditto	Pustules more numerous; along one side was a long continuous band (3 × 1 cm.) of confluent pustules.
		5	Nearly half the surface affected; pustules none.	As above, but with another plum in contact with it also discoloured over the whole surface and bearing many pustules.	Both plums much shrunken and bearing numerous pustules.
TREE II.	*M. cinerea* f. *mali*. Strain from a flowering spur of an apple tree.	1	The rot extended 0·5–1·5 cm. from point of inoculation; no pustules.	Whole surface discoloured and wrinkled, plum shrunken; pustules many. Another plum in contact with it was discoloured over half its surface and bore a few pustules.	Both plums shrunken and bearing many pustules.
		2	Slight discoloration only, immediately round the wound.	Whole surface discoloured but not wrinkled and plum not shrunken; pustules none.	Wrinkled and shrunken; pustules few.
		3	Extension of rot 1·0–1·8 cm.; no pustules.	Whole surface discoloured, plum a little shrunken; pustules many.	Wrinkled and shrunken; pustules many.
		4	Extension of rot 1·0–1·5 cm.; no pustules.	As in No. 3, but pustules few.	Wrinkled and shrunken; pustules few.
		5	Extension of rot 1·3–1·5 cm.; no pustules.	As in No. 3; pustules many.	Wrinkled and shrunken; pustules many.

	Strains used in the inoculation. (*Aug.* 1).	*Plum No.*	*Aug.* 6.	*Aug.* 13.	*Aug.* 28.
TREE II.	*M. cinerea* f. *pruni.* Strain from a mummied cherry.	1	Half the surface affected; no pustules.	Whole surface discoloured and wrinkled, plum shrunken; pustules numerous. One other in contact discoloured over the whole surface but bearing no pustules.	Both plums shrunken and bearing numerous pustules.
		2	One-third of the surface affected; no pustules.	Whole surface discoloured; shrinkage slight; pustules many.	Wrinkled and shrunken; numerous pustules.
		3	Nearly half the surface affected; a few pustules.	As in 2.	As in 2.
		4	Nearly half the surface affected; no pustules.	Whole surface discoloured, plum shrunken; pustules numerous.	Fallen.
		5	One-third of the surface affected; no pustules.	As in 4.	Fallen.

On August 28 some of the plums on these and adjoining trees were ripe; thus those affected from contact with plums artificially inoculated must have become infected just as they were ripening, so that the observations covered the period from the time the plums were quite small (i. e. June 21, when they were not half grown) to their maturescent period. In the experiment started on August 1 (results tabulated) there is evidence that on plums infected near the time of ripening the apple form of *Monilia cinerea* produces fewer pustules than the *Prunus* form; the difference, however, is not striking and both forms readily cause infection of other plums in contact with those primarily infected. The experiments therefore afford no clear explanation of the rare occurrence (perhaps absence) of the form *mali* on naturally infected plums.

The experiment is interesting as illustrating the rapidity with which the rot traverses infected plums. Thus in most cases one-third to one-half the surface was discoloured on the fifth day after inoculation, with the development of pustules in two instances, while on the twelfth day not only were all the primarily inoculated plums affected throughout, but others in contact with these were often also discoloured over their whole surface and bore pustules.

It may here be pointed out that experiments carried out at Wye suggest that in this country plums are infected by the 'Brown Rot' fungi only through wounds. Inoculations made in 1917 by placing conidia of

Monilia fructigena and *M. cinerea* on the uninjured skin of the immature fruit gave negative results. A similar result was obtained in 1918 when conidia of the strain of *M. cinerea* obtained from a plum (the strain used in the experiment just described) were placed on the uninjured skin of 12 young Monarch plums (not half grown) under conditions favourable for their germination, i.e. a gentle rain was falling at the time and the atmosphere was saturated with moisture for at least 12 hours after the inoculations were made. A few days later 12 Victoria plums were similarly treated, and again no infection occurred. Valleau (1915), working in America with the form of *Monilia* which is found there, obtained different results, for he finds 'that infection may take place through the uninjured skin at any time during the development of the plum fruit'.

(*c*) *Plum Flowers inoculated with Strains of Monilia cinerea.*

During the month of April, 1918, a series of three experiments was carried out on flowers of plum trees in the open, the number of flowers inoculated altogether being 39.

The strains of *Monilia* used in each experiment were:

M. cinerea f. *mali*, strain from a Brown Rot Canker of an apple tree.

„ f. *pruni*, strain from a mummied plum.

„ f. *pruni*, strain from a mummied cherry.

The inclement weather which prevailed during the latter half of April removed many of the inoculated flowers at an early stage in the experiments, but a sufficient number remained long enough for the following conclusions to be drawn.

All three strains were equally able to cause infection of the flowers. Each strain was able not only to kill the inoculated flower but to invade the axis of the inflorescence and so cause the death of all the flowers at that node. In two of the experiments, in which the conidia were placed on the stigmas, the results were similar to those already published (1918) for inoculations carried out in 1917 on plum flowers with a 'Wither Tip' strain of *Monilia cinerea*, except that the progress of the disease, particularly in the early stages, was retarded, this being in all probability due to the lower temperature. The first symptom of infection after inoculation of the stigma is a brown discoloration of the stigma itself, usually within two days; this is followed by an extension of the browning downwards along the style, which can be followed day by day until the ovary is reached and soon the whole flower is affected.

In the third experiment the conidia were placed inside the flowers so that the disc became inoculated; in this case the first external evidence of infection was the appearance of a brown patch at one side of the calyx tube. Since the method adopted in making the inoculations and the results

obtained in the early stages of infection in this experiment were different from those of previous experiments the details are given in full.

Small particles of potatoes bearing conidiophores were cut from a pure culture of the fungus growing on sterilized potato and taken to the garden in a sterilized Petri dish, a separate dish being used for each strain. One of the particles was inserted, on the point of a sterilized needle, within the flower in such a way that the conidiophores came in contact with the disc and deposited conidia. The potato particle was then replaced in the dish and another taken for the next flower. Flowers were selected in which there was sufficient space between the stamens and ovary to allow of this operation being carried out without injuring the organs.

The inoculations were made on flowers of Monarch plum trees in the College plantation on April 12; twelve flowers were inoculated, four with each of the three strains.

On April 21 no difference could be detected between the inoculated flowers and others in the vicinity except in the case of one flower (No. 4, inoculated with the cherry strain), the stamens and style of which were withering. Three days later, however, on most of the inoculated flowers there was evidence that infection had occurred.

Strains used in inoculations.	*Result, April 24.*
M. cinerea f. *mali.* Strain from a 'Brown Rot' Canker of apple.	1. No discoloration to be detected. 2. Slight browning on inner surface of calyx tube 3. No discoloration. 4. No discoloration.
M. cinerea f. *pruni.* Strain from plum.	1. Ovary and *base* of style brown; one side of calyx tube brown on inner and outer surfaces. 2. Inner surface of calyx tube brown on one side. 3. One side of calyx tube brown on both surfaces. 4. No discoloration.
M. cinerea f. *pruni.* Strain from cherry.	1. Slight discoloration on inner surface of calyx tube. 2. One side of calyx tube brown on both surfaces. 3. Slight discoloration on inner surface of calyx tube. 4. One side of calyx tube brown on both surfaces; style and stamens brown.

When the flowers were next examined, May 7, all had been blown off except two, viz. No. 2 inoculated with the apple strain, and No. 3 with the cherry strain. The former by this time had become withered to the base and the disease had invaded the flowering axis and caused infection of a second flower (also withered) inserted there, while the latter showed a similar result, but in this case both infected flowers bore grey *Monilia* pustules.

The wilting of plum flowers may be produced therefore by inoculation of either the stigmas or the floral disc with conidia of *Monilia cinerea.*

(*d*) *Apple Flowers inoculated with Strains of Monilia cinerea.*

In the course of the investigation of the 'Wither Tip' disease of plum trees it was discovered that two morphologically similar strains of *Monilia cinerea* had the power to infect apple flowers in different degrees of intensity. A strain obtained from a diseased plum twig, although it was able to induce withering of the actual flowers inoculated, was unable to proceed beyond the base of these, and the rest of the flowers of the same inflorescence were not affected, while a strain obtained from an apple spur not only readily infected apple flowers but invaded the tissues of the axis of the inflorescence and caused a wilting of all the flowers and leaves borne on that axis (Figs. 10 and 11). These results suggested the occurrence of 'Biologic Forms' within the species *Monilia cinerea*; for confirmation of this conclusion further experiments were carried out in 1918, using other strains of *M. cinerea*.

One of the strains selected had been isolated from a tree of *Pyrus japonica* which was affected with a Blossom Wilt resembling that of the apple. This strain, though obtained from a species of the same genus as the apple, showed certain physiological characters similar to those of strains isolated from species of *Prunus*. It had been isolated from a wilted flower in July, 1917, and when used for inoculations had been cultivated saprophytically on artificially prepared media for ten months. In the experiment in which it was used, a strain obtained from an apple spur in March, 1917, was taken for comparison.

The first experiment of the series was carried out with one strain of the apple form of *Monilia cinerea* originally isolated from a 'Brown Rot' Canker in 1916, but re-isolated in 1917 and again in 1918 from an apple spur which had been infected after artificial inoculation of a flower with a pure culture in 1916. Thus in 1918 the original and two sub-strains were available for the experiment, viz. (1) the original strain which had been growing on artificial media for two years, (2) sub-strain grown for about twelve months artificially, (3) sub-strain isolated a few weeks previous to the inoculations. The experiment was carried out with the object of ascertaining whether the form of *M. cinerea* which causes the Blossom Wilt of apples retains its virulence after continued culture on artificially prepared media.

The strains used in this series of experiments on apple flowers were from various sources and hosts as shown below:

Strain A_1.—Isolated in April 1916 from a 'Brown Rot' Canker of a Cox's Orange Pippin apple tree (specimen sent from Berkshire), and afterwards cultivated in pure cultures in the laboratory.

Strain A_2.—Isolated in March 1917 from an apple spur which had become infected in the College plantation after artificial inoculation in 1916 with a pure culture of Strain A_1.

Strain A_3.—Obtained from the same spur as A_2, but cultures started in March, 1918.

Strain B.—From a naturally infected spur of a Lord Derby apple tree in the College plantation in April, 1918.

Strain C.—From a dead plum twig in the College plantation, isolated March, 1918.

Strain D.—From a mummied plum sent from Cambridgeshire, March, 1918.

Strain E.—From a mummied cherry sent from Mid-Kent, March, 1918.

Strain F.—From a flower of *Pyrus japonica* from Mid-Kent, July, 1917.

The experiments were all carried out on apple trees of the James Grieve variety growing in the plantation. Spurs, each bearing an umbel of several flowers, were labelled and two flowers (*a* and *b*) in each umbel were inoculated. The situation of the inoculated flowers on the spur was noted so that a comparison could be made between inoculated and non-inoculated flowers on each umbel, the latter thus serving as controls during the early stages of infection. The inoculations were made as in the experiment on plum flowers, except that in the present case the conidia-bearing surface of the potato particles was brought in contact with the stigmas.

Experiment 1.

Strains used { A_1 Apple Blossom Wilt strain, isolated 1916.
A_2 " " " 1917.
A_3 " " " 1918.

The inoculations were made on May 8, 1918, and the results to May 21 are given in the accompanying table:

Strain.	*Spur.*	*Flower.*	*May* 14.	*May* 18.	*May* 21.
A_1	1	a.	Styles brown for 1–8 mm.	All the flowers of the inflorescence dead; leaves at the base of the umbel wilting.	The whole inflorescence, together with leaves at base, was now brown and dead; flowers recurved and leaves curled.
		b.	Stigmas only brown.		
	2	a.	Styles brown to base.	Ditto	Ditto
		b.	Ditto		
	3	a.	Ditto	Both inoculated flowers had brown withered stamens; pedicels brown and wilting; some of the leaves were wilting, but the non-inoculated flowers were upright.	Ditto
		b.	Ditto		

Strain.	*Spur.*	*Flower.*	*May* 14.	*May* 18.	*May* 21.
A_2	1	a.	Styles brown to base.	Brown to base of pedicel; stamens and calyx lobes brown.	Both flowers dead; leaves beginning to wilt.
		b.	Ditto	Stamens and calyx lobes brown and withered.	
	2	a.	Ditto	Both flowers brown and withered to the base of pedicels.	Whole inflorescence dead; flowers brown, withered, and recurved; leaves brown and curled.
		b.	Ditto		
	3	a.	Ditto	Both flowers brown and withered. The other flowers on this spur and the leaves at base were just beginning to wilt.	Ditto
		b.	Ditto		
A_3	1	a.	Two styles with brown stigmas only, the others brown to base.	Both flowers had brown withered stamens.	Both flowers brown and withered to base of pedicel.
		b.	All the five styles brown to base.		
	2	a.	Styles brown for 1–4 mm.	Both flowers brown to base of pedicels.	Whole inflorescence dead; flowers and leaves brown and withered.
		b.	Styles brown to base.		
	3	a.	Two styles with brown stigmas only, the others brown for 1–4 mm.	Both flowers withered; stamens and calyx brown.	Ditto
		b.	Styles brown to base.		
Controls.			Flowers not inoculated on these and on other spurs of the same tree had, generally, brown stigmas only, but a few showed a slight browning of the styles also.	The rest of the flowers had brown styles, but the stamens were upright, with white filaments, and the calyx lobes were green and spreading.	On the rest of the tree the flowers at this time were just 'setting' into fruit.

On May 25 the two flowers of spur No. 1 inoculated with strain A_3 had fallen; the spur itself was not affected and one of the non-inoculated flowers was 'setting' into fruit. The fungus had by this time advanced some distance into the tissues of the other infected spurs, as distinctly shown in those cases where the spur was branched and bore two inflorescences, one of which bore inoculated flowers; the non-inoculated umbel and the accompanying leaves were withered, thus indicating that the transpiration current had been interrupted at its junction with the main axis of the spur, which therefore must have been invaded by the fungus

travelling downwards from the other (infected) umbel. To bring about this result the fungus must have traversed a distance of from 4·5 to 5·5 cm. between the time of inoculation, on May 8, and May 25.

This experiment proves conclusively that the virulence of the apple Blossom Wilt form of *Monilia cinerea* is not diminished after from one to two years' culture as a saprophyte on artificially prepared media in the laboratory. It also shows that the conidia produced on a spur during the second year after infection occurs are as virulent as those produced during the first year subsequent to infection.

EXPERIMENT 2.

Strains used:
- B. Apple Blossom Wilt strain, isolated 1918.
- C. A 'Wither Tip' strain from a plum tree, isolated 1918.
- D. From a mummied plum, isolated 1918.
- E. „ „ cherry, „ „

The inoculations were made on May 9, 1918.

It will be seen from the table that during the first six days after inoculation the symptoms of infection were generally similar for all the four strains ; later the apple Blossom Wilt strain made rapid progress and caused, in the case of two spurs, the death of the whole inflorescence and the leaves borne on the spur, while with the other strains the disease was confined to the flowers actually inoculated, i. e. the tissues of the spurs were not invaded and the leaves were unaffected.

On May 15 a comparison of the inoculated and the non-inoculated flowers showed that in the former infection had occurred in every case ; this, together with the fact that of the eighteen flowers inoculated with strains C, D, and E all had fallen except two, leads to the conclusion that these strains either kill the flowers directly or at least infect the styles and so prevent pollination, the final result being the same, viz. the arrest of any further development of the flowers, which in consequence wither and fall off. The one instance in which the ovary did begin to swell probably means that pollination had occurred in that case before the inoculation was made.

Strain.	*Spur.*	*May* 15.	*May* 19.	*May* 22.	*May* 28.
B.	1	a. Styles brown to base. b. Ditto	Both flowers brown and withered to base of pedicel; calyx lobes withered.	All the flowers and leaves on the spur brown and withered.	Cortex brown to base of spur, i.e. for 5 cm. from the insertion of the flowers; a vegetative shoot on same spur dead.
	2	a. Styles brown for 3–6 mm. b. ,, ,, 4–6 mm.	Withered to base of pedicel. Stamens and calyx brown and withered.	Flower dead. Fallen.	Both fallen.
	3	a. Styles brown for 6–10 mm. b. ,, ,, 1–2 mm.	Withered to base of pedicel. Stamens and calyx brown and withered.	Both withered to base of pedicel.	All flowers and leaves on the spur dead; a vegetative shoot on the same spur wilting.
C.	1	a. Styles brown for 2–10 mm.	Styles brown to base.	Flower withered to base of pedicel. Stamens brown and collapsed; three calyx lobes withered.	Both flowers fallen; two others of the same umbel are alive and the leaves show no signs of wilting.
		b. ,, to base.	Half the stamens and two calyx lobes brown.		
	2	a. Styles brown to base.	Stamens brown.	Stamens brown and collapsed; calyx lobes withering. Flower withered to base of pedicel.	All the flowers fallen; leaves healthy.
		b. Ditto	Stamens and calyx lobes brown.		
	3	a. Ditto	Half the stamens brown.	Stamens brown and collapsed; calyx lobes withered. Flowers withered to base of pedicel.	Both flowers fallen; one other flower on the same spur had developed into a fruit 1 cm. in length; leaves healthy.
		b. Ditto	Stamens and calyx lobes brown.		
D.	1	a. Styles brown for 3–4 mm.	Indistinguishable from controls.	Indistinguishable from control flowers. Stamens brown and collapsed; calyx lobes withering.	Both dead; one other flower on same umbel alive; leaves healthy.
		b. ,, 8–10 mm.	Stamens wilting.		
	2	a. ,, 3–6 mm.	Indistinguishable from controls.	Indistinguishable from control flowers.	Flower dead.
		b. ,, 3–5 mm.	Ditto	Ditto	Developing into fruit.
	3	a. ,, 3–5 mm.	Stamens and calyx brown, also 0.5 cm. of pedicel. Indistinguishable from controls.	As on May 19. Pedicel brown for 1 cm. below ovary.	All flowers of the umbel fallen; leaves healthy.
		,, 3–8 mm.			

Experiment 2 (*continued*).

Strain.	*Spur.*	*May* 15.	*May* 19.	*May* 22.	*May* 28.
E.	1	a. Styles brown for 5–10 mm. b. ,, ,, 1–5 mm.	Styles brown to base; stamens beginning to wilt; calyx lobes brown at tip.	Stamens brown and collapsed.	Both flowers fallen; two others on same spur forming fruit; leaves healthy.
	2	a. ,, ,, 1–6 mm. b. ,, ,, 3–10 mm.	Both indistinguishable from controls.	Indistinguishable from controls.	a. Flower alive but ovary not swollen. b. Flower dead.
	3	a. ,, ,, 8–10 mm. b. ,, to base.	Ditto	a. Flower withered to base of pedicel. b. Indistinguishable from controls.	Both flowers fallen; one other flower on the same spur had developed into a fruit 1 cm. long; leaves healthy.
Controls.		Flowers not inoculated generally with brown stigmas only; a few showed a slight discoloration of the style.	Styles brown to base, but stamens upright with white filaments; calyx lobes green and spreading.	Controls generally with upright stamens and white filaments, tips of calyx lobes brown; a few flowers had set into fruit —these have brown stamens.	Healthy flowers and mostly developed into fruit up to 1 cm. in length.

Experiment 3.

Strains used { A_2. Apple Blossom Wilt strain, isolated 1917.
F. Strain from *Pyrus japonica*, isolated 1917.

The inoculations were made on May 11, 1918.

Strain.	*Spur.*	*May* 17.	*May* 21.	*May* 24.	*May* 30.
A_2.	1	a. Styles brown for 9–10 mm. b. „ to base.	Styles of both brown to base, but stamens upright and calyx lobes green and spreading as in controls.	Both flowers fallen; one other flower on the spur developing into fruit.	Fruit of developing non-inoculated flower now 1 cm. in length.
	2	a. „ „ b. „ „	Both flowers withered to base of pedicels; stamens brown and collapsed: the rest of the flowers on this spur also dead and leaves wilting.	All the flowers of the spur withered on brown recurved pedicels; all the leaves brown, withered, and curled.	Cortex of spur brown and dead for 3 cm. below the insertion of the flowers.
F.	1	a. „ for 4–6 mm. b. „ „ 5–6 mm.	Both flowers brown and withered to base of pedicels; the other flowers on the spur alive and healthy.	Both inoculated flowers fallen.	Fruit of a non-inoculated flower on this spur 1 cm. in length.
	2	a. „ to base. b. „ for 4–10 mm.	Styles brown to base in both; filaments of stamens upright.	a. Stamens brown and withered. b. Stamens wilting.	a. Withered to base of pedicel. b. Fallen.
	3	a. „ „ 8–10 mm. b. Two styles with brown stigmas only. Three styles brown for 2–5 mm.	Styles brown to base in both; stamens and calyx as in controls.	a. Stamens brown and withered. b. Stamens and calyx lobes brown and withered.	a. Withered to base of pedicel. b. Fallen.
	4	a. Styles brown for 7–10 mm. b. „ „ 6–10 mm.	As in controls.	Stamens brown and withered in both; one other flower on same spur developing into fruit.	a. Dead b. Fallen One non-inoculated flower forming fruit 1 cm. in length.
Controls.		Flowers not inoculated show generally no discoloration at all or a browning of the stigmas only, but in a few instances styles were brown for 1 mm.	Styles usually brown to base; stamens upright with white filaments; calyx lobes green and spreading.	Generally with stamens withering, but many still with white filaments; some flowers developing into fruit (ovary swelling).	Controls, in those cases where the fruit has 'set', have fruit about 1 cm. in length.

As in Expt. 2 the brown discoloration of the styles of all the inoculated flowers on the sixth day after the commencement of the experiment showed that infection had taken place. All these flowers failed to develop into fruit; in the case of one of the spurs infected with the apple strain the typical Blossom Wilt condition was produced, the whole inflorescence together with the leaves on the spur becoming withered within ten days. In this experiment the strain from *Pyrus japonica* proved to be less virulent than the apple Blossom Wilt strain, and is therefore more nearly allied, biologically, to the form of *Monilia cinerea* found on plums and cherries.

Experiment 4.

Strains used { C. A 'Wither Tip' strain from a plum tree.
D. From a mummied plum.
E. „ „ cherry.

The inoculations were made on May 11. This experiment was made to supplement Expt. 2, the three strains being the three *Prunus* strains used on that occasion.

Strain.	*Spur.*	*May* 17.	*May* 21.	*May* 24.	*May* 30.
C.	1	a. Styles brown to base. b. Ditto	Not distinguishable from controls.	Both fallen.	No fruit developing from any of the flowers, but spur alive and leaves healthy.
	2	a. Ditto b. Ditto	Ditto	a. Fallen. b. Developing into fruit.	b. Fruit 1 cm. long.
D.	1	a. Styles brown for 2–5 mm. b. One style brown for 6 mm., the other four have brown stigmas only.	Not distinguishable from controls.	a. Stamens turning brown. b. Fallen.	Both inoculated flowers fallen one other has developed into fruit 1 cm. long.
	2	a. Styles brown for 4–7 mm. b. „ „ 2–3 mm.	Ditto	a. Fallen. b. Fallen.	All the flowers on this spur fallen, but leaves healthy.
E.	1	a. „ „ 3–10 mm. b. „ to base.	Both flowers brown and withered to base of pedicels.	No further change noticeable.	All the flowers fallen, but leaves healthy.
	2	a. „ „ b. „ for 5–7 mm.	a. Ovary brown; stamens brown and collapsed; calyx lobes withering. b. Styles brown to base.	Both fallen.	Two non-inoculated flowers have formed fruit 1·5 cm. long.
Control.		Flowers not inoculated generally show no discoloration or a browning of stigmas only; occasionally a style is brown for 1 mm.	Styles mostly brown to base; stamens not yet withered; calyx lobes green.	Most flowers with withered stamens, but many have still white upright filaments.	In those flowers where the fruit is 'setting', it is about 1 cm. in length.

Again observations on the sixth day after inoculation showed that infection of the styles had occurred. As before, one inoculated flower developed into fruit; the rest all became withered and fell off.

The results of these four experiments, together with those obtained in the experiment carried out in 1917, may be summarized as follows:

RESULTS OF INOCULATIONS MADE ON APPLE FLOWERS WITH STRAINS OF *MONILIA CINEREA*.

			No. of flowers inoculated.	*No. of spurs on which the inoculated flowers were situated.*	*No. of spurs killed.*	*No. of inoculated flowers which developed into fruit.*
'Blossom Wilt and Canker' strains of *Monilia cinerea* from apple trees	1918	A_1	6	3	3	0
		A_2	10	5	4	0
		A_3	6	3	2	0
		B	6	3	2	1
	1917	A_2	4	4	4	0
	Total . .		32	18	15	1
Other strains of *M. cinerea* from various sources	1918	Plum	10	5	0	1
		Cherry	10	5	0	0
		'Wither Tip' of plum	10	5	0	1
		Pyrus japonica	8	4	0	0
	1917	'Wither Tip' of plum	10	4	0	0
	Total . .		48	23	0	2

Thus of the 18 spurs bearing flowers which had been inoculated with the strains from apple trees, 15 were killed outright, with all their flowers and leaves, while the inoculation of 48 flowers with strains from sources other than the apple did not produce in any one instance infection of the tissues of the spur, although there was evidence that infection of the flowers actually inoculated had occurred.

The conclusion is, that the 'Blossom Wilt and Canker Disease' of apple trees is caused by a specialized form of *Monilia cinerea*.

Woronin (1900) stated, as a result of his own inoculation experiments on apple flowers, that *M. cinerea* was unable to produce a Blossom Wilt of apple trees. The host from which he obtained the strain used in his experiments is not stated, but in all probability it was a cherry or plum; had he worked with a strain taken from a dead spur or canker of an apple tree he might have obtained a different result.

VI. CONCLUSIONS.

A general discussion of the results recorded in the present article is reserved for a future occasion, when they will be correlated with those of

certain physiological and cultural experiments carried out with the same fungi. It will be convenient, however, to summarize briefly the results of the observations and experiments described in the preceding pages, as follows:

1. In this country two distinct species of *Monilia* occur as parasites on fruit trees, viz. *M. fructigena*, Pers., and *M. cinerea*, Bon.

2. Each species has two forms distinguished by the effects produced on mature apples inoculated under laboratory conditions.

3. The morphological species *Monilia cinerea* includes two 'biologic' forms, one of which produces a 'Blossom Wilt and Canker Disease' of apple trees, and another which is unable to cause infection of the apple inflorescence other than the flower actually inoculated.

Bibliography.

1796. Persoon, C. H.: Observationes Mycologicae, p. 26. Lipsiae, 1796.
1801. ——————: Synopsis Methodica Fungorum, p. 693. Gottingae, 1801.
1805. Albertini, J. B. de, und Schweinitz, L. D. de: Conspectus Fungorum in Lusatiae superioris Agro Niskiensi crescentium, No. 1090, p. 365. Lipsiae, 1805.
1817. Kunze, G., und Schmidt, J. C.: Mycologische Hefte, i. 80, 1817.
1818. Ehrenberg, C. G.: Sylvae mycologicae berolinensis. Berlin, 1818.
1822. Persoon, C. H.: Mycologia Europaea, p. 24. Erlangae, 1822.
1829. Fries, E.: Systema Mycologicum, iii. p. 430. Gryphiswaldae, 1829.
1830. Duby, J. E.: Botanicon Gallicum, Pt. II, p. 932. Paris, 1830.
1833. Wallroth, F. G.: Flora Cryptogamica Germaniae, p. 182. Norimbergae, 1833.
1844–53. Rabenhorst, L.: Deutschlands Kryptogamenflora, p. 37. Leipzig, 1844–53.
1851. Bonorden, H. F.: Handbuch der allgemeinen Mykologie, p. 76. Stuttgart, 1851.
1864. B(erkeley), M. J.: Gard. Chron., vol. xxxv, p. 818, Aug. 27, 1864.[1]
1869. Fuckel, L.: Symbolae Mycologicae, p. 348. Wiesbaden, 1869.
1871. Cooke, M. C.: Handbook of British Fungi, p. 604 (No. 1812), 1871.
1873. Saccardo, P. A.: Mycologiae Venetae, p. 117. Patavii, 1873.
1875. Thümen, F. von: Der Grind oder Schimmel des Obstes, *Oidium fructigenum*. Oesterr. Landw. Wochenblatt, pp. 41–8, 1875.
1876. Hallier, E.: Eine Pilzkrankheit des Steinobstes. Wiener Obst- und Gartenztg., p. 1272, 1876.
1879. Thümen, F. von: Fungi pomicoli. Monographische Beschreibung der auf den Obstfrüchten der gemässigten Climate vorkommenden Pilze. Wien, 1879.
1881. Peck, C. H.: *Oidium fructigenum*, Knz. and Schm. Fruit Oidium. 34th Annual Rept. N. Y. State Mus. Nat. Hist. (1880), pp. 34–6.
1885. Smith, W. G.: Gard. Chron., vol. xxiv, new series, pp. 51–2, July 11, 1885.
1886. Arthur, J. C.: Rotting of Cherries and Plums. *Oidium fructigenum*, S. and K. New York State Agr. Expt. Sta., 4th Ann. Rept. (1885), pp. 280–5, 1886.
—— Saccardo, P. A.: Sylloge Fungorum, iv. 34, 1886.
1887. Thümen, F. von: Die Pilze der Obstgewächse. Wien, 1887.
1888. Briosi, G., e Gavara, J.: I funghi parassiti delle piante coltivate od utiles, fasc. v, No. 110, *Ovularia necans*. Pass., 1888.

[1] I wish to thank the Editors of the Gardeners' Chronicle for sending me a typewritten copy of this article.

1888. SORAUER, P.: Die Schäden der einheimischen Kulturpflanzen durch thierische und pflanzliche Schmarotzer, p. 235. Berlin, 1888.
1889. GALLOWAY, B. T.: Brown Rot of the Cherry. *Monilia fructigena*, Pers. U. S. Dept. Agr. Rept. for 1888, pp. 349–52.
—— SMITH, ERWIN F.: Peach Rot and Peach Blight (*Monilia fructigena*, Persoon). Jour. Mycol., vol. v, 1889, pp. 123–34.
1891. DRATHEN, VON: Schleswig-Holsteinsche Zeitschrift für Obst- und Gartenbau, 1891, p. 53.
—— HUMPHREY, J. E.: The Brown Rot of Stone Fruits (*Monilia fructigena*, Pers.). Eighth Ann. Rept. Mass. Agric. Expt. Sta. for 1890, Public Document No. 33, Jan. 1891, p. 213.
—— SMITH, ERWIN F.: Peach Blight. Jour. Mycol., vol. vii, 1891, No. 1, pp. 36–8.
—— WITTMACK: Jahresbericht des Sonderausschusses für Pflanzenschutz. Arbeiten der Deutschen Landwirtschafts-Gesellschaft, p. 213, 1891.
1892. CHESTER, F. D.: Can Peach Rot be controlled by Spraying? Delaware Agr. Expt. Sta. Bull., 19, pp. 1–16, 1892.
—— JONES, L. R.: The Brown Rot of Plums. Ver. Agr. Exp. Sta. Rept., No. 5, pp. 134–5, 1892.
—— PRILLIEUX, M.: Sur une maladie du cognassier. Bull. de la Soc. bot. de France, 1892, pp. 209–12.
1893. HUMPHREY, J. E.: On *Monilia fructigena*. Bot. Gaz., 1893, pp. 85–93.
—— MASSEE, G.: British Fungus Flora, vol. iii, p. 283. London, 1893.
—— SCHRÖTER, C.: Kryptogamen-Flora von Schlesien, Bd. iii, p. 67. Pilze, 1893.
1894. BAILEY, L. H.: Impressions of the Peach Industry in Western New York. N.Y. (Cornell) Agr. Expt. Sta. Bull., 74, pp. 379–81, 1894.
—— TAFT, L. R.: Peach and Plum Culture in Michigan. Mich. Agr. Expt. Sta. Bull., 103, pp. 55–6, 1894.
—— WITTMACK and FRANK: Gartenflora, 1894, p. 302.
1895. MASSEE, G.: British Fungus Flora, vol. iv, p. 281, London, 1895.
—— WEHMER, C.: Beiträge zur Kenntnis einheimischer Pilze. Jena, 1895.
—— WORTMANN, J.: Einige Beobachtungen über den Grind des Obstes, hervorgerufen durch *Oidium fructigenum*. Ber. d. kgl. Lehranstalt für Obst-, Wein- und Gartenbau zu Geisenheim a/Rh. für das Etatsjahr 1894–95, pp. 64–7.
1896. WEHMER, C.: Pilzkrankheiten land- und forstwirthschaftlicher Kulturgewächse im Hannoverschen während des Sommers 1896. Cent. f. Bakt., 2. Abt., Bd. ii, 1896, p. 780.
1897. ADERHOLD, R.: Über die in den letzten Jahren in Schlesien besonders hervorgetretenen Schäden und Krankheiten unserer Obstbäume und ihre Beziehungen zum Wetter. Bot. Abt., Versuchs. d. Königl. Pomolog. Instituts zu Proskau, Dec. 1897.
—— ————: Proskauer Obstbau-Zeitung, 1897, p. 108.
—— ————: Zur Monilia-Epidemie der Kirschbäume. Gartenflora, 1897, pp. 429–33.
—— FRANK, B., und KRÜGER, F.: Die Monilia-Epidemie der Kirschbäume. Ibid., p. 320 and p. 393.
—— GOFF, E. S.: The Culture of Native Plums in the North-west. Wisconsin Agr. Expt. Sta. Bull., 63, pp. 18–19, 1897.
—— KINNEY, L. F.: The Plum Rot and its Effect on Plum Culture in Rhode Island. Rhode Island Agr. Expt. Sta., 9th Ann. Rept. (for 1896), pp. 191–2.
—— PRILLIEUX, E.: Maladies des plantes agricoles et des arbres fruitiers et forestiers causées par des parasites végétaux, vol. ii, 1897.
—— WORONIN, M.: Kurze Notiz über *Monilia fructigena*, Pers. Zeitschr. f. Pflanzenkr., Bd. vii, 1897, pp. 196–8.
—— ZSCHOKKE, A.: Ueber den Bau der Haut und die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte. Landwirtschaftliches Jahrbuch der Schweiz, Bd. xi, 1897, p. 153 et seq.
1898. BEHRENS, J.: Beiträge zur Kenntnis der Obstfäulnis. Cent. f. Bakt., 2. Abt., Bd. iv, 1898, p. 514 et seq.
—— BIEMÜLLER: Das Beschneiden der vom Pilz befallenen Kirschbäume, insbesondere der 'Ostheimer Weichsel'. Gartenflora, 1898, p. 107.
—— FRANK, B.: Massregeln gegen die Monilia-Krankheit der Kirschbäume. Ibid., pp. 47–9.
—— ————: Zur Bekämpfung der Monilia-Krankheit der Obstbäume. Ibid., p. 617.
—— ————: Massregeln gegen die Monilia-Krankheit der Kirschbäume. Deutsche landwirtschaftl. Presse, 1898, No. 9, p. 95.

1898. FRANK, B., und KRÜGER, F.: Der Ueberwinterungszustand der Kirschbaum-Monilia. Gartenflora, 1898, p. 96.
—— GOETHE, R.: Bericht d. kgl. Lehranstalt für Obst-, Wein- und Gartenbau zu Geisenheim a. Rh. für das Etatsjahr 1897–8. Wiesbaden, 1898.
—— REUTER, E.: In Norwegen im Jahre 1896 aufgetretene Krankheitserscheinungen. Zeitschr. f. Pflanzenkr., Bd. viii, 1898, p. 209.
—— WEHMER, C.: Die Monilia-Krankheit (*Monilia fructigena*) unserer Obstgärten. Beilage zur Hannoverschen Land- und Forstwirthschaftlichen Zeitung, 1898, Nos. 3 and 8.
—— ————: *Monilia fructigena*, Pers. (= *Sclerotinia fructigena*), und die Monilia-Krankheit der Obstbäume. Berichte der Deutschen Botan. Gesellsch., Bd. xvi, 1898, p. 298.
—— WORONIN, M.: *Monilia cinerea*, Bon., und *Monilia fructigena*, Pers. (Vorläufige Mittheilung). Botan. Centralblatt, Bd. lxxvi, 1898, pp. 145–9.
1899. ADERHOLD, R.: Proskauer Obstbau-Zeitung, iv. Jahrg., 1899, pp. 20, 55, 83, 115.
—— BEHRENS, J.: Kupferpräparate und *Monilia fructigena*. Cent. f. Bakt., 2. Abt., Bd. v, 1899, pp. 507–9.
—— CORDLEY, A. B.: Brown Rot (*Monilia fructigena*, Pers.). Oregon Agr. Expt. Sta. Bull., 57, 1899.
—— FRANK, B., und KRÜGER, F.: Ueber die gegenwärtig herrschende Monilia-Epidemie der Obstbäume. Landwirtsch. Jahrb., Bd. xxviii, 1899, pp. 185–216.
—— MONTEMARTINI, L.: La *Monilia fructigena*, Pers., e la malattia dei frutti da essa prodotta. Estr. d. Riv. di patol. veget., 1899, vol. viii, pp. 210–18.
—— SCHELLENBERG, H. C.: Ueber die Sclerotinienkrankheit der Quitten. Berichte der Deutschen Bot. Gesellsch., Bd. xvii, 1899, pp. 205–15.
—— SORAUER, P.: Erkrankungsfälle durch *Monilia*. Zeitschr. f. Pflanzenkr., Bd. ix, 1899, pp. 225–35.
—— ————: Zur Monilia-Krankheit. Berichte der Deutschen Bot. Gesellsch., Bd. xvii, 1899, pp. 186–9.
1899–1900. ————: Erkrankungsfälle durch *Monilia*. Zeitschr. f. Pflanzenkr., Bd. ix, 1899, pp. 225–35; Bd. x, 1900, pp. 148–54, 274–84.
1900. ADERHOLD, R.: Zwei gefährliche Erkrankungsfälle unseres Kernobstes. Prosk. Obstbau-Ztg., Jahrg. v, 1900, pp. 39–42.
—— ————: Eine dem 'fireblight' Amerikas äusserlich ähnliche Krankheit des Apfelbaumes. Cent. f. Bakt., 2. Abt., Bd. vi, pp. 628–9.
—— MÜLLER-THURGAU, H.: Die Monilienkrankheit oder Zweigdürre der Kernobstbäume. Ibid., pp. 653–7.
—— ————: Die Monilienkrankheit oder Zweigdürre der Kernobstbäume. Schweizer Zeitschr. f. Obst- u. Weinbau, Nos. 13, 14, 1900, pp. 198–203.
—— QUAINTANCE, A. L.: The Brown Rot of Peaches, Plums, and other Fruits (*Monilia fructigena*, Persoon). Georgia Exp. Sta. Bull., 50, Oct. 1900, pp. 237–69.
—— SORAUER, P.: Schutz der Obstbäume gegen Krankheiten, p. 125, 1900.
—— WEHMER, C.: Pilzkrankheiten von Kulturpflanzen in der Provinz Hannover. Cent. f. Bakt., 2. Abt., Bd. v, pp. 55–6.
—— WAUGH, F. A.: Plum-tree Canker. Vermont Agr. Expt. Sta. Rept., No. 13, pp. 370–3.
—— WORONIN, M.: Über *Sclerotinia cinerea* und *Sclerotinia fructigena*. Mém. Acad. Imp. Sci. St. Pétersbourg, viii. sér., vol. x, No. 5, Phys. Math., pp. 1–38.
1902. CLINTON, G. P.: Apple Rots in Illinois—Brown Rot. Ill. Agr. Exp. Sta. Bull., 69, p. 190.
—— MASSEE, G.: Plant Diseases. Jour. Roy. Hort. Soc., vol. xxvi, 1901–2, p. 736.
—— NORTON, J. B. S.: *Sclerotinia fructigena*. Trans. Acad. Sci. St. Louis, vol. xii, pp. 91–7.
—— OSTERWALDER, A.: Die Blüten- und Zweigdürre bei *Cydonia japonica*. Gartenflora, Jahrg. 51.
1903. ALWOOD, W. B., and PRICE, H. L.: Notes on spraying Plums. Virginia Agr. Expt. Sta. Bull. 134, pp. 31–40.
—— BOS, J. RITZEMA: Tijdschrift over Plantenziekten, ix, p. 128.
—— CARD, F. W., and SPRAGUE, L. P.: The Sand Cherry and Fruit Rot. Rhode Is. Agr. Exp. Sta. Rpt. for 1902, pp. 246–7.
—— MASSEE, G.: Text-book of Plant Diseases, p. 300. London, 1903.
1904. ADERHOLD, R.: Über eine vermuthliche zu *Monilia fructigena*, Pers., gehörige *Sclerotinia*, Ber. Deut. Bot. Ges., Bd. xxii, pp. 262–6.

1905. ADERHOLD, R., u. RUHLAND, W.: Zur Kenntnis der Obstbaum-Sklerotinien. Arb. Biol. Abt., Land- u. Forst. Kaiserl. Gesundheitsamte, iv, pp. 427–42.
—— ————————: Die Monilia- (Sclerotinia-) Krankheiten unserer Obstbäume und ihre Bekämpfung. Kaiserl. Biolog. Anst. f. Land- und Forstwirtschaft. Flugblatt, Nr. 14.
—— CLINTON, G. P.: Notes on Fungus Diseases. Conn. Agr. Exp. Sta. Rept. for 1904, pp. 316–17.
1906. SACCARDO, P. A.: *Sclerotinia fructigena.* Syll. Fung., vol. xviii, pp. 40–4.
—— HEALD, F. D.: The Black Rot of Apples due to *Sclerotinia fructigena.* Nebr. Agr. Expt. Sta. Rept., No. 19, pp. 82–91.
—— COOKE, M. C.: Fungoid Pests of Cultivated Plants, p. 135. London, 1906.
1907. FAUROT, F. W.: Brown Rot of Peach. Missouri State Hort. Soc. Rept., 1907, pp. 285–9.
—— SCOTT, W. M.: A Promising Treatment for Brown Rot and other Peach Diseases. Proc. 30th Sess. Amer. Pomol. Soc., pp. 39–45.
—— MOLZ, E.: Ueber die Bedingungen der Entstehung der durch *Sclerotina fructigena* erzeugten 'Schwarzfäule' der Aepfel. Cent. f. Bakt., 2. Abt., Bd. xvii, pp. 175–88.
—— LINDAU, G.: Rabenhorst's Kryptogamen-Flora, i^{8}. 52.
—— SALMON, E. S.: 'Brown Rot' of Acid Cherries. Jour. S. E. Agric. Coll., Wye, Kent, No. 16, pp. 283–6.
1908. DANDENO, J. B.: Winter Stage of *Sclerotinia fructigena.* Rept. Mich. Acad. Sci., vol. x, pp. 51–3.
—— READE, J. M.: Preliminary Notes on some Species of *Sclerotinia.* Annales Mycol., vi. 109–15.
—— SCHELLENBERG, H. C.: Untersuchung über das Verhalten einiger Pilze gegen Hemizellulosen. Flora, Bd. xcviii, pp. 257–308.
—— SORAUER, P.: Handbuch der Pflanzenkrankheiten, Bd. ii, pp. 288–91.
1909. POLLOCK, J. B.: Notes on Plant Pathology. 11th Rept. Mich. Acad. Sci., pp. 51–4, 1908–9.
—— SCOTT, W. M., and AYRES, T. W.: The Control of Peach Brown Rot. Georgia State Hort. Soc. Proc., xxxiii, pp. 96–104.
1910. KÖCK, G.: Beobachtungen über den Befall verschiedener Kirschen- und Weichselsorten durch den Moniliapilz (*Sclerotinia cinerea*, (Bon.) Schröt.). Sond., Zeitschr. f. d. landwirt. Versuchsw. in Österr., Jahrg. 13, 1910, p. 889.
—— LEWIS, A. C.: Brown Rot Experiments in 1909. Ga. State Bd. Ent. Bull., 32, pt. 2, pp. 35–48.
—— MASSEE, G.: Diseases of Cultivated Plants and Trees, p. 270. London, 1910.
—— MÜLLER-THURGAU, H.: Die Moniliakrankheit der Apfelbäume. Schweizer Zeits. f. Obst- u. Weinbau, Jahrg. 19, 1910, p. 212.
—— MUTH, F.: Über die Fäulniss der Quitten. Sond., Zeits. f. Wein-, Obst- u. Gartenbau, 1910, p. 162.
—— POLLOCK, J. B.: Notes on *Sclerotinia fructigena.* Abstract in Science, N.S., vol. xxxi, p. 437, 1910.
—— SALMON, E. S.: The 'Brown Rot' Canker of Apple Trees. Jour. S. E. Agric. Coll., Wye, Kent, No. 19, 1910, pp. 355–7.
—— ————————: A 'Canker' of Apple Trees caused by the 'Brown Rot' Fungus. Gard. Chron., vol. xlvii, p. 327, May 21, 1910.
—— SCOTT, W. M., and AYRES, T. W.: The Control of Peach Brown Rot and Scab. U.S. Dept. Agr. Bur. Pl. Ind. Bull. 174, pp. 1–31, 1910.
—— SCOTT, W. M., and QUAINTANCE, A. L.: Control of the Brown Rot and Plum Curculio on Peaches. U.S. Dept. Agr. Bu. Ent. Cir., 120, pp. 1–7, 1910.
—— STEWART, F. C.: Notes on New York Plant Diseases. New York Agr. Exp. Sta. Bull., 328, pp. 338–9, 1910.
1911. SCOTT, W. M., and QUAINTANCE, A. L.: Spraying Peaches for the control of Brown Rot, Scab, and Curculio. U.S. Dept. Agr. Farmers' Bull., 440.
1912. BRUSCHI, D.: Attività enzimatiche di alcuni funghi parassiti di frutti. Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., vol. xxi, pp. 225–30, 298–304.
—— DEMAREE, J. B.: A *Sclerotinia* on Apple. Science, N.S., vol. xxxv, pp. 77–8, 1912.
—— EWERT, R.: Verschiedene Überwinterung der Monilien des Kern- und Steinobstes und ihre biologische Bedeutung. Zeitsch. f. Pflanzenkr., Bd. xxii, pp. 65–86, 1912.
—— GÜSSOW, H. T.: A New Disease of Peaches. Canadian Expt. Farm Rept., 1911, p. 251, 1912.

1912. SORAUER, P.: Weswegen erkranken Schattenmorellen besonders leicht durch *Monilia*? Zeits. f. Pflanzenkr., Bd. xxii, pp. 285–92, 1912.
—— VOGES, E.: Über Monilia-Erkrankung der Obstbäume. Ibid., pp. 86–105, 1912.
—— WESTERDIJK, JOH.: Die *Sclerotinia* der Kirsche. Mededeelingen uit het phytopathologisch Laboratorium 'Willie Commelin Scholten', Bd. iii, pp. 39–41.
—— WHETZEL, H. H.: The Fungous Diseases of the Peach. Proc. New York State Fruit Growers' Assoc., 1912.
1913. BROZ, O.: Die Moniliagefahr. Separatabdruck aus 'Der Obstzüchter', Nr. 7.
—— COOK, M. T.: Report on the Plant Pathologist for 1912. New Jersey Agric. Expt. Sta.
—— ERIKSSON, J.: Zur Kenntnis der durch *Monilia*-Pilze hervorgerufenen Blüten- und Zweigdürre unserer Obstbäume. Mycol. Centralbl., Bd. ii, pp. 65–78.
—— JEHLE, R. A.: The Brown Rot Canker of the Peach. Phytopath., vol. iii, pp. 105–10.
—— MATHENY, W. A.: A Comparison of the American Brown Rot Fungus with *Sclerotinia fructigena* and *S. cinerea* of Europe. Bot. Gaz., vol. lvi, pp. 418–32.
—— RABATÉ, E.: Ann. servant aux Épiphyties, Mém. et Rap., tom. ii, pp. 341–6. (Plum Diseases: Abstract in Exp. Sta. Rec., vol. xxxvi, p. 751, 1917).
—— SALMON, E. S.: The 'Brown Rot' Canker of the Apple. Jour. S. E. Agric. Coll., Wye, Kent, No. 22, pp. 446–9.
1914. CONEL, J. L.: A Study of the Brown Rot Fungus in the Vicinity of Champaign and Urbana, Illinois. Phytopath., vol. iv, pp. 93–102.
—— COOLEY, J. S.: A Study of the Physiological Relations of *Sclerotinia cinerea*, (Bon.) Schroeter. Ann. Missouri Gard., vol. i, No. 3, pp. 291–326.
—— FAES, H.: Terre Vaud., tom. vi, No. 25, pp. 282–3. (A Disease of Apricot in Valais Abstract in Exp. Sta. Rec., vol. xxxv, p. 49, 1916.)
—— HOWITT, J. E.: Notes on the Apothecial Stage of *Sclerotinia cinerea* in Ontario. Ottawa Nat., vol. xxvii, No. 11, pp. 158–60.
—— JEHLE, R. A.: Peach Cankers and their Treatment. Cornell Univ. Agr. Expt. Sta. Circ., 26.
—— ORTON, C. R.: Some Orchard Diseases and their Treatment. Proc. State Hort. Assoc., Penn., vol. lv, pp. 43–56.
—— SALMON, E. S.: The 'Brown Rot' Canker of the Apple. Gard. Chron., Aug. 1, 1914, p. 85.
1915. BAILEY, F. D.: Experimental Spraying of Prunes for Control of Brown Rot. Oregon Agr. Exp. Sta. 2nd Bienn. Crop Pests and Hort. Rept. for 1913–14, pp. 241–4.
—— CAYLEY, D. M.: Brown Rot of Fruit. Gard. Chron., Oct. 30, 1915, p. 269.
—— CHIFFLOT, J., and MASSONNAT: Rev. Hort. (Paris), tom. lxxxvii, No. 27, pp. 540–1. (A Disease of Apricot in Rhône Valley. Abstract in Exp. Sta. Rec., vol. xxxv, p. 50, 1916.)
—— ——————: Compt. Rend. Acad. Agr. France, tom. i, No. 15, pp. 473–7. (Apricot Disease in the Rhône Valley. Abs. in Exp. Sta. Rec., vol. xxxv, p. 249, 1916).
—— DARNELL-SMITH, G. P., and MACKINNON, E.: Fungus and other Diseases of Stone Fruits. Agr. Gaz. N.S. Wales, vol. xxvi, No. 7, p. 589; No. 9, p. 749.
—— JACKSON, H. S.: Prune Brown Rot on Dried Fruit. Oregon Exp. Sta. 2nd Bienn. Crop Pests and Hort. Rept. for 1913–14, pp. 276–7.
—— POSEY, G. B.: Studies of *Monilia* Blight of Fruit Trees. Science, vol. xlii, No. 1086, p. 583.
—— SPINKS, G. T.: A Black Rot of Apples. Ann. Rept. Agric. and Hort. Res. Sta., Long Ashton, Bristol, 1915, pp. 94–6.
—— STAKMAN, E. C., and ROSE, R. C.: Experiments in the Control of Brown Rot of Plums. Minn. Hort., vol. xliii, No. 5, pp. 231–3.
—— VALLEAU, W. D.: Varietal Resistance of Plums to Brown Rot. Jour. Agric. Res., vol. v, pp. 365–95.
—— VOSS, G.: Flugbl.-Samml. ü. Pflanzenschutz, K. Landw. Akad. Bonn-Poppelsdorf, No. 7, p. 4. (*Monilia* on Fruit Trees. Abstract in Exp. Sta. Rec., vol. xxxv, p. 655, 1916.)
1916. BARTRAM, H. E.: A Study of the Brown Rot Fungus in Northern Vermont. Phytopath., vol. vi, pp. 71–8.
—— BROOKS, C., and COOLEY, J. S.: Temperature Relations of Apple Rot Fungi. Jour. Agric. Res., vol. viii, pp. 139–63.
—— BROOKS, C., and FISHER, D. F.: Brown Rot of Prunes and Cherries in the Pacific Northwest. U.S. Dept. Agr. Bull., 368.

1916. HESLER, L. R.: Diseases of the Plum: Brown Rot. New York Agric. Dept. Bul., 79, pp. 1192–5.
—— SPINKS, G. T.: A Black Rot of Apples. Ann. Rept. Agric. and Hort. Res. Sta., Long Ashton, Bristol, 1916, pp. 153–5.
—— MANSFIELD, A. B.: Ripe Rot of Stone Fruits. Jour. Agr. (New Zeal.), vol. xii, No. 3, pp. 214–16.
1917. PEGLION, V.: Atti R. Acad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., 5ª ser., vol. xxvi, No. 12, pp. 637–41. (A Gummosis of Apricot. Abstract in Exp. Sta. Rec., vol. xxxviii, p. 650, 1918.)
—— WORMALD, H.: A Blossom Wilt and Canker of Apple Trees. Ann. Applied Biol., vol. iii, No. 4, April, 1917, pp. 159–204.
—— ——————: The 'Blossom Wilt and Canker' Disease of Apple Trees. Jour. Bd. Agric., vol. xxiv, No. 5, Aug., 1917, pp. 504–13.
1918. COCKAYNE, A. H.: Report of the Biologist: Plant Diseases. New Zealand Dept. of Agric., Industries, and Commerce, Ann. Rept. for 1917–18, p. 38.
—— CUNNINGHAM, G. H.: Brown Rot in Cherries controlled. Jour. Agr. (New Zeal.), vol. xvi, No. 1, pp. 38–39.
—— MCCUBBIN, W. A.: Peach Canker. Dom. of Canada Dept. Agric. Bul. 37, 1918.
—— WORMALD, H.: 'Brown Rot' of Apples. Jour. Bd. Agric., vol. xxv, No. 3, June, 1918, pp. 299–302.
—— ——————: A 'Wither Tip' of Plum Trees. Ann. Applied Biol., vol. v, No. 1, July, 1918, pp. 28–59.
1919. MCCUBBIN, W. A.: Brown Rot of Stone Fruits. Agric. Gaz. of Canada, May, 1919, pp. 429–32.

EXPLANATION OF PLATES XXV AND XXVI.

Illustrating Mr. Wormald's paper on the 'Brown Rot' Diseases of Fruit Trees.

PLATE XXV.

Fig. 1. Plums naturally infected, above with *Monilia cinerea*, the two below with *M. fructigena*.

Fig. 2. Plums naturally infected, the one on the right with *M. fructigena*, the one on the left with both *M. fructigena* (right side) and *M. cinerea* (left side).

Fig. 3. Fruiting spur of apple bearing pustules of *M. fructigena* in late summer; infection occurred through the fruit, the stalk of which is seen at the upper end.

Fig. 4. Apple (var. Bramley's Seedling) inoculated with pure cultures, on the left with *M. fructigena*, on the right with *M. cinerea* f. *mali*: result on seventh day after inoculation. At this stage the dark rings round the lenticels on the side infected with *M. cinerea* f. *mali* are a characteristic feature.

Fig. 5. The apple shown in Fig. 4, but 13 days later; the right side of the apple was quite black by this time.

Fig. 6. Apple inoculated from pure cultures, on the right with *M. cinerea* f. *mali*, on the left with *M. cinerea* f. *pruni*.

Fig. 7. Apple inoculated from pure cultures with two strains of *M. fructigena*, on the left a strain from a plum, on the right a strain from an apple affected with 'Black Rot'.

Fig. 8. On the left a plum inoculated from a pure culture of *M. fructigena*, on the right a plum inoculated from a pure culture of *M. cinerea*. Control plum, from the same tree, in the centre.

Fig. 9. Plum inoculated from pure cultures, on the left with *M. fructigena*, on the right with *M. cinerea*. (The experiments illustrated by Figs. 8 and 9 were carried out in the open, the specimens being removed from the tree immediately before the photographs were taken.)

PLATE XXVI.

Fig. 10. Above, on the left, an inflorescence of an apple tree (var. Lord Derby), of which a single flower had been inoculated with conidia from a pure culture of *M. cinerea* f. *mali*; infection has reached the base of the spur and a canker is just making its appearance on the stem. Result 16 days after inoculation.

Fig. 11. As in Fig. 10, but 5 days later; the canker has nearly girdled the stem.

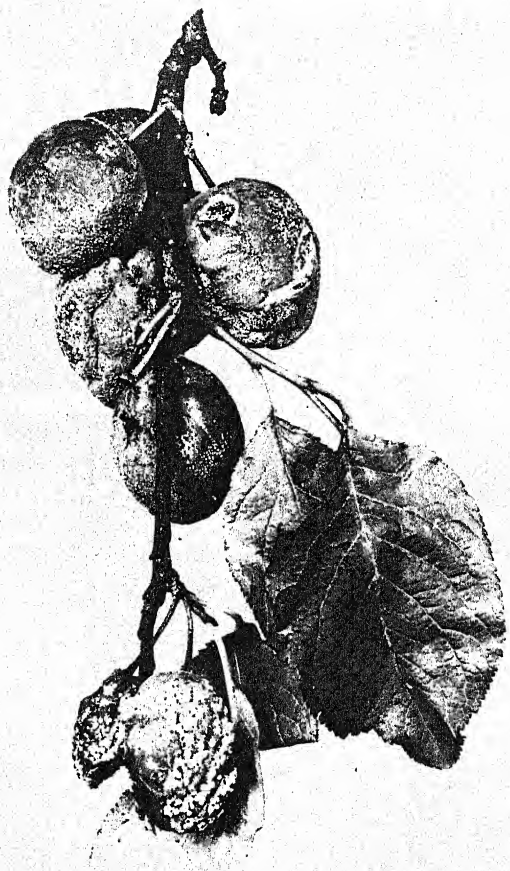
Fig. 12. A canker, from an apple tree infected with *M. cinerea* f. *mali*, bearing pustules during the third year after infection; photographed in January, 1919. (The same canker photographed in 1917, one year after infection, is shown in the Jour. Bd. Agric., Aug., 1917, p. 504, Fig. 1.)

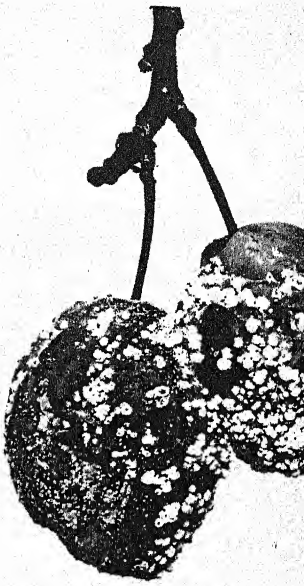
Fig. 13. Dead spur and canker from an apple tree (var. Warner's King) photographed Mar. 22, 1918: result of inoculating a single flower of the inflorescence in May, 1916, with conidia from a pure culture of *M. cinerea* f. *mali*. The canker produced in 1916 is nearly covered by callus, but the spur still bears pustules of viable conidia.

Fig. 14. Dead spur and canker of an apple tree (var. James Grieve), winter condition, the result of the natural infection of the fruit with *M. fructigena* during the previous summer: the fruit-stalks, from which the diseased apples had fallen, are seen at the apex of the spur. The fungus was isolated from barren pustules on the spur and from mycelium in the canker. (Compare Fig. 3, which shows the summer condition of the fungus on an apple spur.)

Fig. 15. Above are two flowering spurs which had become infected by *M. cinerea* f. *mali*, resulting in a canker on the branch. The check to the upward flow of sap stimulated buds below the canker to grow out into weak vegetative shoots.

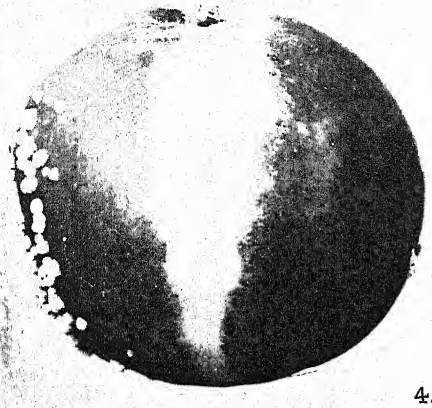
Fig. 16. Section through an infected spur and canker of an apple tree. The lesion on the branch is being covered by callus. × 2.



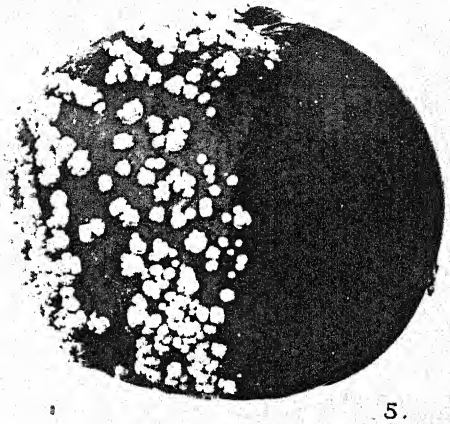


1.

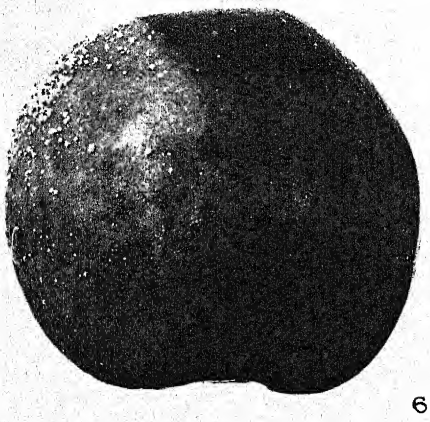
H. WORMALD — MONILIA.



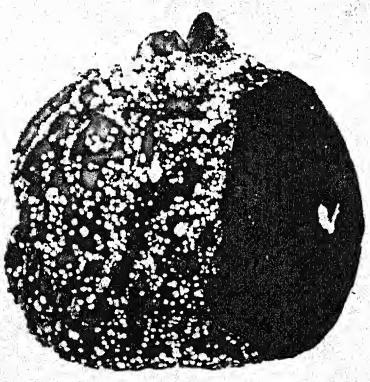
4.



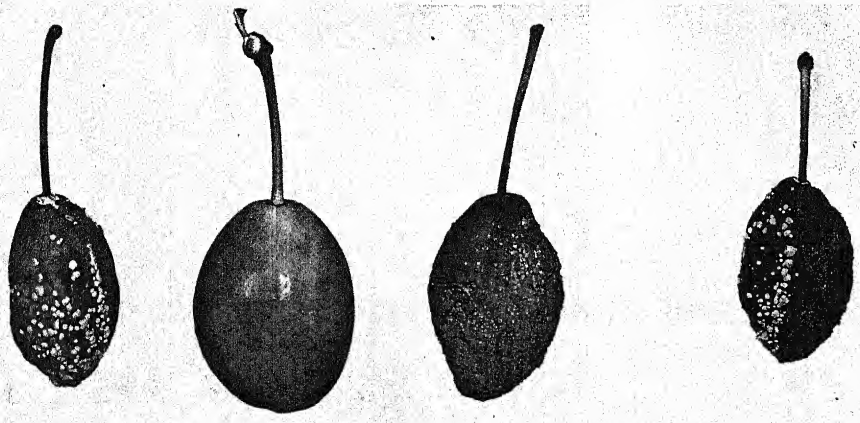
5.



6.



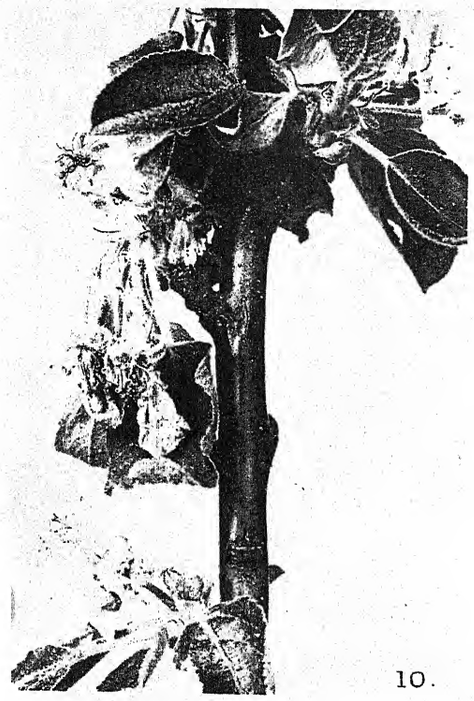
7.



8.

9.

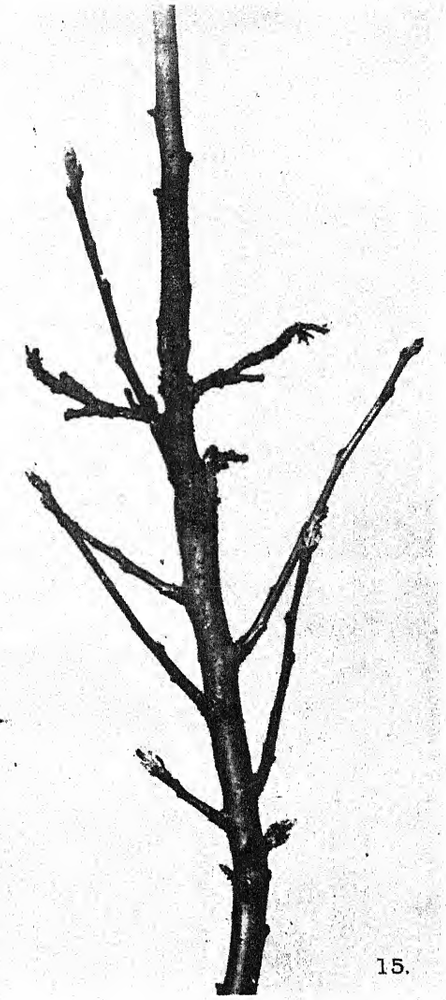
Huth coll.



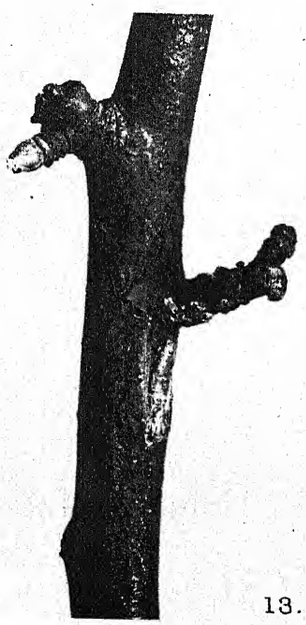
10.

11.

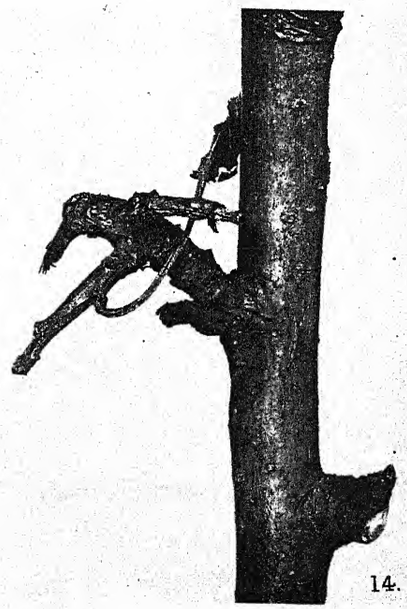
12.



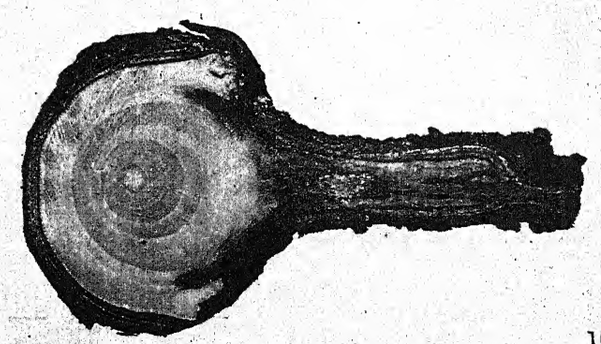
15.



13.



14.



16.

H. WORMALD—MONILIA.

Huth coll.

12. DEC 1919

Mōcharas and the Genus Haematomyces.

BY

T. PETCH,

Government Botanist and Mycologist, Ceylon.

With two Figures in the Text.

THE genus *Haematomyces* was established by Berkeley and Broome in 1873 (Jour. Linn. Soc., xiv, p. 108) on a specimen sent from Ceylon by Thwaites The generic description runs as follows:

'*Haematomyces. Tremelloides, sinuato-lobatus, gyrosus, subcerebrinus, immarginatus; asci vesiculares; sporidia elliptica.*'

The type species was named *Haematomyces spadiceus*, B. and Br. No formal description of the species was given, but it was said to look exactly like a *Tremella*, and to have spores, 6·25 μ long, which bore a small proportion to the wide, obovate, vesicular asci. Berkeley and Broome added the note, 'There can be no doubt that the genus is good; but it is to be hoped that a further supply of specimens will be procured. The asci are quite unlike anything in Bulgaria, to which we were at first inclined to refer it. Black when dry.'

The genus was duly included in Saccardo, 'Sylloge Fungorum,' in the Bulgarieae. Another species had by that time been described by Peck, viz.:

Haematomyces orbicularis, Peck, *Sessilis, pulvinatus, orbicularis, subtremellosus, gyroso-convolutus, nigricanti-brunneus, particulis minutis rufis punctatus; ascis anguste clavatis, apice subacutis; sporidiis oblongo-fusoideis, continuis*, 15–18 × 3–4 μ; *paraphysibus numerosis, filiformibus.*

The generic character on which Berkeley and Broome laid so much stress, viz. the obovate, vesicular asci, was apparently considered of minor importance.

Subsequently Peck added a third species:

Haematomyces fagineus, Peck, *Tremelloides, cerebriformis*, 2–2·5 cm. diam., *gyroso-lobata, glabra, nitens, extus intusque rubra (raisin colour); ascis subcylindraceis*, 8-*sporis*, 60 × 7·5 μ; *paraphysibus gracilibus, supra lenissime incrassatis; sporidiis plerumque monostichis, anguste ellipsoideis*, 7·5 × 3·5–5 μ. *Ad truncos Fagi ferrugineae. Habitus Tremellae.*

[Annals of Botany, Vol. XXXIII. No. CXXXII. October, 1919.]

A fourth species was described by Rick from Brazil:

'*Haematomyces eximius*, Rick, *Ascomate gelatinoso, cerebriformi et tremelloideo, prorumpente, convolutionibus crassis, firmis, globoso, 5 cm. diam., castaneo; ascis cylindraceis*, 130 × 6–8 μ; *sporidiis ellipsoideis*, 6–8 μ *longis*, 4 μ *cr., biguttulatis, apiculatis v. etiam apice truncatis, viridulo-hyalinis, dein olivascentibus, biserialibus, unilocularibus; paraphysibus filiformibus, apice paulatim minute incrassato, hyalinis, versus pedem viridulo-olivaceis.*'

And Rick adds '*Haemat. spadiceo affinis*', a remark which strikingly illustrates the futility of comparisons based on descriptions only.

In 'Fragmente zur Mykologie', VI. Mitt., p. 126, von Höhnel writes, 'Another Discomycete genus with an immarginate disc is *Haematomyces*, Berk. This genus appears to me to be an immarginate *Ombrophila*. Presumably those species of *Psilopeziza* which are brilliantly coloured (*flavida, aurantiaca, xylogena, aquatica*) would be better regarded as species of *Haematomyces*.

'To this genus, no doubt, belongs also *Pezizella orbilioides*, Feltgen ('Vorstud. Pilzflora von Luxemburg,' III. Nachtr., p. 53), which I formerly referred to *Ombrophila*. It must now be named *Haematomyces orbilioides*, (Feltg.) v. H. It corresponds admirably with this genus.'

Apparently the accepted idea of *Haematomyces* is a pulvinate, convoluted, or cerebriform stroma, more or less gelatinous or tremelloid in consistency, which bears a palisade layer of asci and paraphyses over the whole of its exposed surface, without any definite margin. The spores are continuous and hyaline. None of the descriptions states explicitly that the asci are arranged as suggested, but that conclusion appears to be warranted by the inclusion of the genus in the Bulgarieae. Berkeley and Broome made the shape of the asci a generic character, but that has been ignored by all the describers of subsequent species.

Berkeley and Broome's material appears to have been scanty. There is a specimen, part of the original gathering, in the Peradeniya herbarium, but as for twelve years I did not meet with any species which coincided with the current idea of *Haematomyces*, it was not examined. During 1918, however, I collected, in the low country, an ascomycete which formed a pulvinate, cerebriform stroma, up to 1·5 cm. diameter, purple-red to flesh colour, and somewhat subtranslucent and tremelloid. The whole of the exposed surface was covered by a palisade layer of asci. As this species appeared to agree with the description of *Haematomyces*, an examination of the cotype was undertaken.

The cotype in Herb. Peradeniya (Thwaites' 59) was evidently obovate when fresh, about 8 mm. high, and 5 mm. broad. It has become laterally compressed in drying, and slightly folded towards the apex, but it is scarcely 'subcerebrinus'. Its colour is dark purple-brown, and its surface minutely rough. On cutting sections, it is found that *there is no palisade*

layer of asci. The stroma consists of a dense brown cortex, about 100 μ thick, succeeded internally by paler tissue, or by alternate zones of hyaline and brown tissue. This tissue shows numerous lines which appear to be the walls of partially disorganized hyphae. These walls run irregularly for a short distance, usually strongly and closely flexuose, and are then lost in the general mass. But the general arrangement of these walls is concentric, and parallel to the outer surface.

Surface sections show that the exterior is composed of large, more or less polygonal cells, but as these do not appear in the cross-section they are evidently very thin, or have collapsed.

Embedded here and there in the internal tissue are the wide, obovate, vesicular asci noted by Berkeley and Broome. These may reach a length of 100 μ and a breadth of 40 or 50 μ. They contain a varying number of subangular bodies, which are sometimes arranged in a row, but more usually lie in a group on one side of the cell, and occupy, as stated by Berkeley and Broome, only a very small part of it.

It will be evident that this structure is not that of the Bulgarieae, and that, if it were a fungus, *Haematomyces* would have to be classed perhaps in the Plectascineae.

On staining with cotton blue in lactic acid, the whole of the tissue turns pale blue, but it does not swell up to any marked extent. But on staining with iodine, the apparent spores stain black and are evidently starch or amyloid grains. This immediately renders it improbable that the obovate bodies are asci, and that the specimen is a fungus.

On considering the matter further, it occurred to me that *Haematomyces spadiceus* was not rare, but was to be found in abundance at Peradeniya. Moreover, I had frequently examined it, without ever suspecting that it might have been mistaken for a fungus. Fortunately, fresh material happened to be available at the time, and an examination of it confirmed the idea suggested by the type specimen.

An Exudation from *Bombax malabaricum*.

One of the most conspicuous trees at Peradeniya is the Bombax (*Bombax malabaricum*, DC.), the red cotton tree, and most visitors to Peradeniya remember the row of giant trees which borders the road from the station to the Botanic Gardens, especially if they have passed that way in the early part of the year when the ground is covered with its large, red, shuttlecock-like flowers. The tree is a rapid grower, and its wood is brittle. Consequently its huge limbs are frequently wrenched off by the wind, and, as they are of little use except for firewood, they are left lying until they begin to decay. As seven trees of the row referred to stand along the frontage of my garden, I have had several opportunities during the last twelve years of observing the changes which take place in the fallen branches.

After a branch has been lying on the ground for a few weeks, it begins to exude, through cracks in the bark, a yellowish, pasty, somewhat viscid substance. This appears in pulvinate masses, or runs down the side in thick strands, assuming all the varied forms associated with an exudation of gum or resin. If the cortex has been stripped off any part of the branch, this substance emerges from between the wood and the cortex, i.e. from the site of the cambium, and it issues from the same place on the cut end of a fallen branch or stem. It soon acquires a yellow-brown, polished, outer skin, and this increases in thickness and deepens in colour as the exudation ages. Sometimes the skin ruptures under the pressure of the exuding mass, and further projections grow out from the apex of the structure first formed. When old, it is red-brown to purple-brown, usually with a polished surface. As it dries it hardens and contracts, sometimes falling into irregular folds and convolutions, sometimes retaining its shape but becoming hollow. This exudation is *Haematomyces spadiceus*.

The following recent occurrence illustrates its usual mode of appearance. A branch about 35 feet long, and 9 inches in diameter at the butt, was blown down at the beginning of July, during the rains. In falling, the outer half of it lodged in a small tree, so that it stood in a nearly vertical position with the thicker end on the ground. During August, the upper part of the branch, above a height of 20 feet, produced numerous green shoots, while the lower end which rested on the ground began to produce the usual exudation. During dry weather in September, shoots arose from the lower end also, and the condition on September 24 was as follows: The exudation was issuing from cracks in the lowest 4 feet, and the same region bore green shoots up to a length of about 9 inches, but the shoots were situated chiefly in the lowest 2 feet 6 inches. On the next 10 feet above that, the bark was dead, but the uppermost 20 feet bore green shoots, up to a foot in length, without any exudation. During a week's drought at the end of September, the shoots on the upper part began to wither, and the exudation began to issue in small amount from the same region. It would appear from this, and other similar observations, that this exudation is only formed in living cortex. When, on a fallen branch or stem, a bud bursts through the cortex, the yellow mass frequently appears at or near its base.

Mocharas.

This exudation from the Bombax has long been known in India, where it is sold as a drug in the bazaars under the name of Mocharas, or Mochras, or Mocherus, &c. Mocharas means the juice (*ras*) of the Mocha, the latter being the Sanskrit name of the Bombax. The 'Dictionary of the Economic Products of India' describes it as a brown, astringent, gum-like substance, which occurs in the form of light or dark brown tears, often hollow, and much resembling galls. Owing to the usual confusion attending the native

names of drugs, there was formerly some difference of opinion whether Mocharas was obtained from the Bombax or the Areca palm. Birdwood, as quoted by Cooke, favoured the latter, and stated that all his attempts to obtain gum of any kind from Bombax completely failed in Bombay, and he had no hesitation in saying that the red cotton tree afforded no gum whatever. It is, however, now definitely settled that Mocharas is the produce of the Bombax, and Birdwood's failure to obtain anything of the kind from standing trees is quite in agreement with the experience of other observers.

Mocharas was included in the collection of Indian gums and resins submitted to Cooke and is referred to by him on p. 40 of his Report. He quotes from the Pharmacopaeia of India that 'The astringent gummy exudation occurs in opaque, dark-brown, knotty pieces, some presenting a remarkable gall-like appearance, inodorous, of a strongly astringent taste.' Cooke included it in his section on Astringent Gums, and stated that many pounds of it had been broken up, but no evidence had been found that it was an insect gall. 'They are not galls, but gall-like exudations, for there is every appearance of their having exuded, in a manner similar to gum, in a semi-fluid state.'

Some further light was thrown on the subject by an article by B. H. Baden-Powell in the 'Indian Forester', viii (1882), pp. 153–5. The following extract is quoted from his account:

'In my garden at Lahore there are two large and well-grown Bombax trees, which must be as old as the very earliest British resident who came to Lahore, if not earlier. . . . I detached bits of the bark from one of these trees, made incisions, cut into the surface only, and down to the sap-wood, but no gum or any other exudation of any kind appeared. But the other tree, which divides into two stems at about eight or ten feet from the ground, is covered, as to its lower part, with a clustering mass of the lovely "pink coral creeper" (*Antigonum leptopus*). Having occasion to trim this, and remove the old stems or bine, I disclosed the surface of the tree, and saw that, close to the fork of the two branches, the stem was somewhat swelled, the bark was all broken up and excoriated, as if in fact a large sort of unhealthy swelling sore was there: a mass of brownish or blackish powder, or rather friable grains, had also fallen and collected at the foot of the tree. This residue looked rather like the excreta of some insects boring into the bark, but I could not detect any trace whatever of any bark-eating insects, or any aphides, &c. A great deal of this powdery stuff was to be brushed off the wood itself, seeming to come from the disintegration of the bark over the sore. From all parts of this sore I found great masses of the Mochras which had exuded and dried there: much of it was old and partly rotten, but some that was fresh looked like deep brown bubbles or shells that had irregularly contracted in drying. I cleared away all the powder, and picking out the best bits of Mochras to go to Dr. Cooke, I cleared away the

old rotten stuff. After a few days, I observed new Mochras form: to my surprise it issued in various shaped masses, or worm-like pieces, as if one squeezed oil paint out of a tube; this gradually curled up or coagulated into a mass as chance would have it. It consisted of a rather firm, slightly translucent, dirty whitish-yellow jelly.

'To the taste it was almost insipid, but with a slight roughness indicating astringency. It proved wholly insoluble in cold water, and nearly so in boiling water, though I think it went into a pulp under such treatment. It did not appear, either, soluble in pure spirits of wine, but imparted a red colour to the liquid.

'This jelly, when dried by the air and heat of the sun, acquired a dark brown colour; the surface dried first, and the inner part gradually shrunk afterwards, accounting for the blister-like irregular pieces. This then is Mochras (at least one kind of it).'

The above experience differs from that of other observers in that the exudation was obtained from a living, though damaged, tree. But it agrees with others in that wounding the tree did not induce its formation.

Dymock, in the 'Materia Medica of Western India', gives the following information regarding Mocharas:

'When first exuded it is a whitish fungous mass which gradually turns red, and finally dries into brittle mahogany-coloured tears. The larger tears are hollow in the centre, the cavity being produced during the gradual drying of the jelly-like mass which first exudes. Dry Mocharas when soaked in water swells up, and resumes very much the appearance of the fresh exudation. The taste is purely astringent, like tannin.

'Mocharas is not a simple juice, but the product of a diseased action, which consists in a proliferation of the parenchyme cells of the bark. Upon making a section of the diseased part a number of small cavities are seen which contain a semi-transparent jelly-like substance, consisting of oblong cells with botryoidal nuclei. At the margin of the cavity the columns of healthy cells are seen breaking up, and the cells separating to join the jelly-like mass: this gradually increases in size and finds its way to the surface to be extruded as Mocharas. Upon its first appearance it is of an opaque, yellowish-white colour, firm externally, but semi-fluid internally, and there is no central cavity. The cause of the diseased condition of the bark which produces Mocharas has not been determined.'

Dymock further states that Mocharas only exudes from portions of the bark which have been injured by decay or insects.

The majority of observers have recorded that they have not been able to induce the formation of this exudation by wounding a healthy tree. There is some possibility of error in these observations, owing to the peculiar character of the Bombax cortex. On a well-grown tree, the cortex at the base of the stem is very thick, and the inner layers readily

separate into thin sheets. When a piece of cortex is cut out, it may split off along one of the inner layers, and unless the exposed tissue is examined microscopically, one is very apt to mistake the smooth, white, cortical layer for the surface of the wood. It appears to be essential for the formation of Mocharas that the wound, on a living tree, should extend to the cambium.

The following observations were made at Peradeniya on the foregoing point, during October, 1918:

A rectangular piece of cortex, about 6 cm. by 4 cm., was cut out, down the wood, from the stem of a young tree about 30 cm. in girth. The cortex was 6 mm. thick. No Mocharas was formed.

Fourteen young stems, of about the same girth as the last, were pollarded at a height of about 4 feet. In one case, a small quantity of Mocharas emerged, about six weeks afterwards, at one point on the cut surface, from between the wood and the cortex. In another, the pollarded stem produced new shoots near the apex, one of which was wrenched off later, and a small quantity of Mocharas was subsequently produced where the young shoot had been torn off.

A piece of cortex, about 5 cm. square, was cut out of the stem of a well-grown tree at a height of 4 feet, the girth of the tree at that height being 12½ feet. The thickness of the cortex was 35 mm. About three weeks afterwards, Mocharas emerged in small quantity from the line of the cambium, the total amount not exceeding a cubic centimetre. It issued chiefly along the lower edge of the opening.

Mocharas, therefore, can be obtained by wounding standing trees, but the quantity is very small in comparison with the amount which issues from fallen branches or felled logs.

The Structure, etc., of Mocharas.

At Peradeniya, Mocharas occurs on sound branches which have been broken off by the wind, or on felled Bombax, in either case after they have been lying on the ground for a few weeks. It issues as a yellowish-white pasty mass, soft and only slightly viscid. The surface soon becomes yellow and forms a thin, polished skin, while the inner part remains, for some time, whitish, subtranslucent, and soft. If it is broken, the inner tissue rapidly turns brown. Finally, it dries and hardens, becoming red-brown to purple-brown: sometimes the outer skin collapses and the mass becomes irregularly folded, but in other cases it retains its shape and becomes hollow internally.

When examined in an early stage, the soft, internal tissue is found to be composed of large cells, either spherical, 40–90 μ in diameter, or more generally oval, up to 130 × 100 μ. These are thin walled and readily collapse. Each of them contains a varying number of small, subangular,

starch (or amyloid) grains, from half a dozen to over fifty, usually in a group towards one side of the cell. As a rule the cells are free from one another, but sometimes a few may be united in pairs. They are bound together by a comparatively small quantity of a finely granular slime. The external covering consists merely of an outer layer of the same cells, browned, collapsed, and glued together by the hardened slime: it may attain a thickness of 100 μ.

If the cortex from which Mocharas is issuing is stripped off the wood, a thin layer of the same substance is usually found lying between the two. This layer is not continuous, but is interrupted by small islands of tissue from one to five millimetres in diameter, or sometimes by large continuous sheets. There are also other smaller masses of tissue, projecting from the surface of the wood, or the inner surface of the cortex, but lying beneath the slimy layer. These patches are usually more numerous on the cortex than on the wood. On exposure the slime rapidly turns brown.

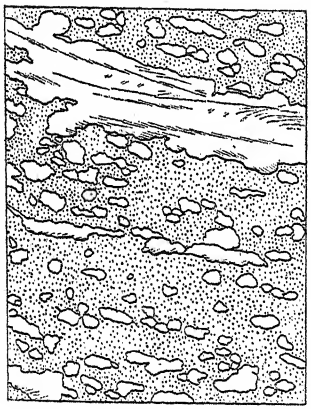


FIG. 1. Inner surface of the cortex of *Bombax malabaricum*, when producing Mocharas. The white areas are unaltered cortical tissue; the intervening shaded spaces are the galleries. Natural size.

On cutting out a piece of the cortex, with the underlying wood still attached, the following conditions are found: Immediately external to the wood is a layer of parenchyma, consisting of rectangular cells arranged regularly in radial rows. This layer may be 2 mm. thick. The cells do not contain any of the sphaerocrystals which are a prominent feature of normal Bombax cortex, and the layer lacks the lignified fibres which occur so abundantly in the outer normal tissue. Instead of the latter, there are usually two, sometimes three, longitudinal bundles of lignified vessels, often running somewhat irregularly, with scalariform thickenings. These vessels, like the normal Bombax cortical fibres and stone cells, give the usual lignin reaction with phloroglucin. They may run singly through the parenchyma, or be united in bundles of two or three. Sometimes they bear a slight resemblance to the vessels of the Bombax wood, though much smaller in diameter, but as a rule they are distinctly scalariform. The walls of this parenchyma do not stain with Sudan glycerine: a number of globules in the cells take the stain, but this occurs also in normal cortex. The walls are stained yellow-brown by chlor-zinc-iodide: they do not stain with phloroglucin.

No layer corresponding to this is found in normal Bombax cortex. In the latter, the cortex consists chiefly of rows of lignified fibres, with narrow

bands of parenchyma up to 6·5 mm. wide. The cells of the normal cortical parenchyma contain large numbers of starch grains, and many contain large sphaero-crystals.

The examination of this abnormal cortex and the exudation has been rather of a preliminary character, as it was undertaken chiefly to determine whether Mocharas was a fungus or not. Many interesting, and apparently novel, features await further investigation.

When the cortex is exuding Mocharas, this tissue is permeated by numerous galleries parallel to the cambium and anastomosing freely. In an advanced stage, the cortex is in some places separated from the wood, or divided tangentially by cavities which leave only a few layers of cortical parenchyma overlying the cambium, while in other places it is united to the wood by narrow columns of parenchyma. Hence when it is stripped away from the wood it presents the irregular appearance already described.

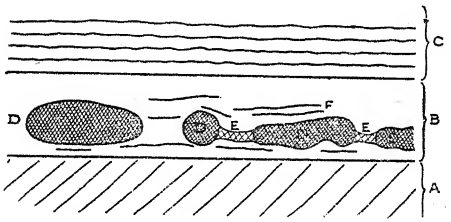


FIG. 2. Longitudinal section of the cortex of *Bombax malabaricum*, when producing Mocharas. Diagrammatic. A, wood; B, abnormal cortex; C, normal cortex with lignified fibres; D, galleries of Mocharas; E, browned tissue, showing extension of Mocharas formation; F, lignified vessels.

The formation of Mocharas begins with the disorganization of a small group of parenchymatous cells, and extends until a gallery is formed. The gallery, in section, may be circular, but is more usually narrow-oval, or oblong with rounded ends, the long axis of the gallery being parallel to the cambium. In the cases observed, this has generally begun about the middle of the abnormal cortical zone.

The radial rows of unaltered cortical cells meet the long flatter sides (i. e. the outer and inner) of the gallery perpendicularly, but at the curved boundary they may be displaced so as to be normal to the curve. The narrow radial boundary of the gallery usually abuts on a normally radial row of cells, and in many cases a wedge-shaped mass of browned cells stretches from this boundary through the parenchyma, parallel to the cambium, indicating an early extension of the gallery in that direction. All the cortical cells round the gallery are in a state of rapid division by cross walls parallel to its surface, except, in some cases, those which border on the shorter sides where the gallery is in process of extension laterally.

The first appearance noted in the cells which are undergoing transformation into Mocharas is a browning and swelling of the cell-walls.

This swelling appears to take place in the inner layers, i. e. those bordering on the lumen of the cell. At the same time hyaline globules appear on the inner surface of the cell-wall and increase in number until in some cases they almost fill the cells: frequently these globules form short chains, or are united in botryoidal masses. The cells then separate along the middle lamella, and intercellular spaces are formed which become filled with slime. The loose cells lose their brown colour, and their walls are reduced to a thin membrane and collapse. These collapsed cells, embedded in slime, completely fill the gallery with a disorganized mass.

The globules on the cell-walls, or in the cells, at first stain yellow-brown with chlor-zinc-iodide. They do not stain with Sudan III. But in the older modified cells, they stain black with chlor-zinc-iodide. This change appears to take place from the centre of the globule, as many of them stain black in the centre, with a yellow-brown translucent margin. It thus appears that these starch (or amyloid) bodies are formed from some constituent of the cell-wall. The grains observed in the cells of the exuded Mocharas are not the residual starch of the cortical parenchyma. On staining a section of the abnormal cortical layer with chlor-zinc-iodide, it is very noticeable that while innumerable granules in the disorganized mass stain black, nothing of the kind occurs in the surrounding unaltered parenchyma. There is no starch in the unaltered cells of the abnormal cortical layer.

A similar formation of Mocharas takes place in the old normal cortex in small pockets between the fibres, but this occurs only to a small extent, and it does not seem to contribute much to the amount exuded. The chief source of the exudation is the abnormal layer of cortical parenchyma which is developed after the tree is felled. Consequently, Mocharas exudes principally along the line of the cambium, or from cracks which extend almost completely through the cortex.

The lignified vessels of the abnormal cortical layer do not form part of Mocharas. They are pushed to one side, as the galleries are enlarged by the disorganization of the surrounding cells.

When the mass exudes, the cells expand and assume an oval or spherical shape. A few are brown, with brown contents, but the majority are hyaline, and contain a cluster of amyloid bodies. No crystals occur in the exudation. The red-brown or purple-brown coloration of the outer layer appears to be produced not only by exposure to the air, but also by a transfer of some colouring matter from the interior outwards. When first exuded, Mocharas is pale yellow, or yellowish white, but when the outer layer has turned red brown, the interior appears distinctly whiter.

When a piece of Mocharas, about three or four weeks old, was boiled in water, it swelled up and resumed its original shape. At the same time its colour changed to yellow, and the water became deep red. When left

in water, the inner part swelled further, burst the outer wall, and issued in mucous-like masses.

When Mocharas from the same sample as above was immersed in cold water, it swelled up and resumed its original shape more gradually. The colour of the outer layer remained unchanged, but the water was nevertheless tinged brown. After twenty-four hours, the outer layer had split and the inner part was emerging in white masses. Twenty-four hours later, these extruded masses had swollen still further and their boundaries were translucent.

The reactions yielded by this substance with different reagents vary in some degree with its age or condition. For example, when it is two or three days old and has acquired a thick, outer, brown coat the behaviour of the internal white mass is not quite the same as that of freshly exuded material, which is covered merely by a thin yellow-brown skin. The differences, however, are in degree only.

In water, material taken from the interior of a piece two or three days old swells up slightly into a translucent jelly in which the individual cells are visible, more or less in lines, like the ova of some aquatic animal. The water is coloured brown almost immediately. The external brown layer does not undergo any appreciable change. The same phenomena occur when this material is immersed in a 5 per cent. solution of cane sugar, but the liquid usually is not coloured and the swelling is generally greater.

When freshly exuded material is immersed in water, it spreads out in an opaque, cream-coloured, slimy layer over the bottom of the tube, the water being coloured brown as before. Some part of the original mass may retain its shape, but the bulk of it spreads out over the base of the tube, instead of swelling up into a definite, pulvinate, translucent mass, as in the case of the older material. In a day or two, the slime swells still more and extends up the tube, driving before it the brown liquid, but still leaving an opaque, creamy layer at the base. In 5 per cent. cane sugar solution the same happens, but the liquid in this case is tinged brown, though only slightly, and more of the original mass remains coherent than in water.

If the freshly exuded material is fixed to the side of the tube, in water, the bulk of it falls to the bottom and forms a creamy layer as before, but some of it remains adherent to the side, and swells into translucent strands in which the cells are embedded. No difference has been observed in the reactions of these two portions, except as regards the starch grains. Those in the cells which sink to the bottom turn evidently blue in chor-zinc-iodide, but those in the cells in the strands take at first a brownish tinge, before becoming opaque. Treated in the same way in 5 per cent. sugar solution, comparatively little falls to the bottom of the tube, the bulk swelling into a cream-coloured mass still adherent to the side.

Alcohol, ether, and chloroform have no action on either fresh or old material.

In 10 per cent. caustic potash, the slime swells up and dissolves. In small quantity, on a slide, the material turns red-brown, but when immersed in bulk it becomes purple. The slime dissolves, and the liquid becomes a deep wine colour. Alcohol discharges the colour, and causes a cloudiness which, however, disappears on standing. If the liquid is neutralized with hydrochloric acid, the colour changes to yellow, and no cloudiness occurs when alcohol is added.

With chlor-zinc-iodide, both the slime and the cell-walls stain yellow-brown. Occasionally a small patch of the slime stains violet. After forty-eight hours in 10 per cent. caustic potash, the cell-walls stain violet, and the starch grains purple-brown to reddish-brown.

Neither the slime nor the cell-walls dissolve in copper-oxide-ammonia.

In strong chromic acid both the slime and the cells dissolve, with evolution of gas from the former. With fresh material, the cell walls are dissolved in about two hours, but after being soaked in water for forty-eight hours they dissolve in a few minutes.

There is no effervescence with hydrochloric acid, sulphuric acid, or acetic acid. Dilute hydrochloric acid induces a granular deposit in the cells which renders them opaque. No calcium crystals are formed with either hydrochloric or sulphuric acid, or with ammonium oxalate after heating with 2 per cent. hydrochloric acid.

With methylene blue, both the slime and the cells stain blue, not violet. Acetic acid does not destroy the colour. Alcohol discharges the colour very slowly: after an immersion of twenty-four hours in 80 per cent. alcohol, the cell-walls have lost all colour, but the slime remains green. Immersion for the same length of time in glycerine leaves both the slime and the cells pale blue.

Ruthenium red stains the cell-walls red, but does not stain the slime.

Alcoholic alkannin does not stain the slime or the cells after an immersion of eighteen hours.

With Sudan III in glycerine, numerous granules in the slime stain red, but the cell-walls and the general mass of the slime remain uncoloured. Immersion for twenty-four hours in dilute alcoholic Sudan III, transferring to glycerine, and heating, as recommended by Küster, only stains the granules as before.

No coloration is produced by phloroglucin.

Aniline blue stains the slime blue or greenish blue; eosin stains it red.

The coloration of the cell-walls in chlor-zinc-iodide, before and after treatment with caustic potash, and their insolubility in copper-oxide-

ammonia indicate that they are suberised. On the other hand, they dissolve in chromic acid, rapidly if previously soaked in water, and they do not stain with alkannin or Sudan III.

The coloration of the slime with methylene blue is not that of pectic compounds and it is not discharged by alcohol or glycerine. The slime does not stain with ruthenium red. It would appear that it is not a pectose slime.

A small amount of the slime stains blue with chlor-zinc-iodide, and is probably cellulose slime, but the main mass is not, as it stains yellow-brown with chlor-zinc-iodide and is insoluble in copper-oxide-ammonia.

The slime gives the reactions for callose in that it is insoluble in copper-oxide-ammonia, readily soluble in caustic potash, and stains, though scarcely typically, with aniline blue. But it also stains with eosin, which does not stain callose: and it does not dissolve in concentrated cold sulphuric acid.

Mocharas is rich in tannin. Ferric chloride produces a green coloration, potassium bichromate an intense red-brown, and ammonium molybdate yellow.

I regret that I am unable to institute any comparisons with the results of previous investigations of the slimes which occur in the Bombacaceae or Malvaceae, as no literature on the subject is at present accessible.

I have not been able to find any evidence that the formation of Mocharas is induced by the action of fungi or bacteria.

When recently exuded, Mocharas is usually quite free from bacteria, or fungus spores or hyphae. Pieces taken from the interior of the mass with the customary precautions showed no growth when placed in culture flasks in (*a*) water, (*b*) a solution containing 5 per cent. cane sugar and 1 per cent. asparagin, or (*c*) standard nutrient solution. Agar plates of (*b*) and (*c*) gave the same result.

Mocharas, according to the evidence obtained, is derived from an abnormal layer of cortical parenchyma which is formed in the stems of *Bombax malabaricum* after they have been felled, or in some instances, though apparently rarely, after the cortex has been injured. It consists of the remains of the walls of the cortical cells, which retain their continuity though much reduced in thickness, bound together by a slime.

HAEMATOMYCES.

Haematomyces spadiceus, B. and Br., is dried Mocharas. The supposed disorganized hyphae seen in a section are the walls of the collapsed and distorted cells, while the few cells which retain, more or less, their original oval shape constitute the 'obovate, vesicular asci'. The bodies which Berkeley and Broome considered spores are amyloid grains.

The genus *Haematomyces*, as originally described, consequently falls, for even if it should subsequently be demonstrated that Mocharas owes its origin to the action of a fungus, the exudation itself, which is the material on which the genus was founded, is not a fungus. The three species of *Haematomyces* since described bear no relation to the original species, if their descriptions can be relied upon.

The simplest way out of the difficulty which thus arises would appear to be that the generic description of *Haematomyces* should be amended in accordance with the ideas which have been associated with the name by Peck, Rick, and mycologists in general; and, though the writer is aware that he is venturing on very dangerous ground in classifying fungi from their descriptions only, the following is proposed:

Haematomyces (Char. emend.). Stroma superficial, pulvinate, often cerebriform or convoluted, tremelloid or fleshy-waxy, bearing a palisade layer of asci and paraphyses over the whole exposed surface, immarginate: spores continuous, hyaline.

The genus appears to be out of place in the Bulgarieae, and should probably be placed in the Helvellaceae. In many points it appears to be too close to *Psilopezia*, Berk.

The following new species has recently been collected in Ceylon:

Haematomyces carneus, n. sp. Pale purple-red to flesh colour, pulvinate, cerebriform, up to 1·5 cm. diameter, superficial, subtranslucent, tremelloid. Asci cylindric, 160 × 10–12 μ, not operculate, eight-spored, spores obliquely uniseriate. Paraphyses stout, inflated at the apex, diffluent. Spores hyaline, oval, thick walled, ends subtruncate, 15–18 × 8–9 μ, exceptionally 26 × 10 μ. On dead wood, leaves, &c. Delwita, Ceylon, June 1918; No. 5767 in Herb. Peradeniya. The whole ascus stains blue with iodine, the spores and paraphyses staining yellow.

Summary.

1. Mocharas is an exudation from wounded or, more commonly, felled *Bombax malabaricum*. It is formed from a special layer of cortical parenchyma, and consists of the remains of cortical cells united by a slime.

2. This substance was made the type of a new genus of fungi, *Haematomyces*, by Berkeley and Broome in 1873, the type species being named *Haematomyces spadiceus*.

3. As the substance is not a fungus, Berkeley and Broome's genus falls, but the name is here retained for those species which have been placed in the genus by subsequent authors.

4. A new species of *Haematomyces* is described.

LITERATURE.

1. BADEN-POWELL, B. H. : Mochras, an Exudation from the Bark of *Bombax malabaricum*. Indian Forester, vol. viii, 1882, pp. 153-5.
2. COOKE, M. C.: Report on the gums, resins, oleo-resins, and resinous products in the India Museum, or produced in India. London, 1874.
3. Dictionary of the Economic Products of India. Article Bombax.
4. DYMOCK, W. The Vegetable Materia Medica of Western India. Bombay, n.d. (1883).

The Origin and Meaning of Medullary (Intraxylary) Phloem in the Stems of Dicotyledons.

II. Compositae.

BY

W. C. WORSDELL.

With twenty-seven Figures in the Text.

INTRODUCTORY.

IN the issue of this journal for October, 1916, the writer set forth the essential features of the vascular structure of the axial and foliar organs of the Cucurbitaceae, showing that this structure is fundamentally a 'Monocotyledonous' one, consisting of a system of scattered bundles of which the 'internal-phloem' strands constitute an inner series, having completely lost their xylem.

It can be deduced from the facts presented in this paper that the vascular structure of the stems and leaves of Compositae belongs to the same type, having also been derived from an ancestral scattered or 'Monocotyledonous' system of bundles. For the Compositae represent one of the Natural Orders many members of which exhibit 'internal' or medullary phloem in their stems, and it can be shown that the medullary phloem-strands really represent an innermost series of vascular bundles belonging to the primitive scattered system.[1]

What was stated in the previous paper, viz. that such relatively conservative organs as the mature stem, peduncle, and foliage-leaf are to be studied in connexion with ancestral characters of this kind rather than the seedling stem, is here again emphasized.

It will thus be seen that the view-point from which the subject is envisaged is entirely different from that of any of the authors mentioned below or, indeed, from that of any previous investigator. Hence the treatment of the subject is different, viz. along lines which tend to emphasize chiefly those facts which have more directly and obviously to do with the main thesis. Many details of fact which are inessential to the elucidation of

[1] In the following pages the structure is usually described as seen in transverse section.

[Annals of Botany, Vol. XXXIII. No. CXXXII. October, 1919.]

the principal argument are omitted, while stress is laid upon other facts which most authors, from their standpoint, would probably deem unworthy of notice.

Historical.

Petersen, in a general paper on bicollateral bundles in various orders, describes a number of Compositae amongst the Cichoriaceae which exhibit intraxylary phloem, e.g. *Sonchus*, *Lactuca*, and *Tragopogon*, and mentions the presence of some xylem in connexion with this tissue. He gives a long list of Cichoriaceous genera in which he could find no trace of medullary phloem. *Crepis*, *Leontodon*, *Picridium*, and *Rodigia* are included in this list, in all of which medullary phloem has since been found.

Weiss, in the following year, 1883, contributed an important treatise on the subject. He seems to have been one of the first to show that the bundles of the main vascular system of the stem possessing internal phloem are not true bicollateral bundles in the original acceptation of that term. The result of his investigations is that the medullary phloem of the stem is in every case a leaf-trace.

For instance, with reference to *Scorzonera hispanica* he states: 'that the phloem-bundles to the inside of the protoxylem cannot be regarded as an integral part of the bundle extending in the same radial line outwards is clear, for they arise later, and they are also the continuation in the pith of a leaf-trace occurring in the cylinder in the internode above the node concerned.' With reference to the case of *Lactuca sativa* he held that the two smaller lateral phloem-strands occurring near each protoxylem-group of the bundle of the cylinder are the direct continuation of those occurring opposite a bundle in the leaf-base. Here the medullary phloem is directly a leaf-trace. But other phloem-strands pass into the pith from the ordinary external phloem of the cylinder, and as this phloem has entered the cylinder from a leaf at a higher node, such medullary phloem-strands may be regarded as indirect leaf-traces.

Kruch's lengthy thesis on the medullary bundles of Cichoriaceae affords us the results of by far the most thorough and complete investigation of the matter extant. In Part I he deals with the distribution and diffusion of the strands, whether they occur exclusively at the periphery of the pith, are partly peripheral and partly central, or scattered irregularly and uniformly throughout the pith. In Part II the course of the strands is dealt with; the conclusion, after an intricate and painstaking study, being that, for the most part, the medullary strands of the stem are either the direct continuation of *flower-traces* or of the medullary strands of *branches*, as in *Tragopogon*. In a minority of instances they are leaf and cauline strands, as in *Lactuca*, where there is no medullary phloem in the branches (at least in their basal part) and that of the leaf-bundle enters the pith of the stem when the leaf-bundle joins the vascular ring (as seen in transverse section), while other

medullary strands enter the pith from the vascular ring; and this latter is the case with the bundles of the peduncle.

Peter made an investigation of the genus *Scorzonera* and concluded that the grouping of the species according to the systematists does not agree with that according to anatomical data. He classifies the species into four groups according to the arrangement and structure of the bundles of the stem: (1) Bundles arranged irregularly in several indistinct series or rings, all collateral; (2) Bundles in two indistinct series of unequal-sized bundles, within or without which lie smaller ones; (3) One regular series of bundles; phloem-strands scattered in the pith, with or without xylem; (4) One series of bundles only; no medullary strands; bundles either collateral or bicollateral.

Col, in an important account of the arrangement of the bundles in the stem and leaves of certain Dicotyledons, cited some interesting facts regarding the presence of internal phloem in the leaves and axial organs of some Compositae.

Miss K. Barratt observed in a fragment of an internode of the stem of *Helianthus annuus* the passage of a bundle of the vascular ring into the pith, becoming there an amphivasal bundle. She draws no inferences from this fact. But the present writer has observed many such cases in other plants (cf. *Tolpis*), and regards them all as instances of partial reversion to the primitive scattered disposition of the bundles composing the main vascular system of the stem. It is, perhaps, a more widely-spread phenomenon in the Compositae than is generally known.

Miss E. Whitaker studied the structure of the vascular cylinder of the stem of species of *Solidago*, with special reference to the mode in which the leaf-trace bundles leave the vascular ring. She starts out from the *a priori* standpoint that the woody type of stem is the more primitive in the Compositae. There is a single reference to the subject of the present paper, as follows: 'internal phloem in the leaf-bundles of the cortex is a general feature of the genus and probably of the family. It seemingly perpetuates a condition which was once characteristic of the bundles of the axis.'

Original Observations.

Cichoriaceae.

Tragopogon pratensis, L.

The stem of this plant has frequently been selected to serve as an example of the occurrence of 'internal phloem' immediately within the xylem of many of the bundles of the main vascular system, such bundles being, therefore, as in Cucurbitaceae, of the 'bicollateral' type. As a matter of fact, this particular structure is characteristic of only a certain region, viz. the higher portion of the aerial stem. The object of the present investiga-

tion, a continuation of that undertaken for the Cucurbitaceae, is to discover the meaning and origin of these internal-phloem strands.

The method adopted has been to make transverse sections, successive and continuous where necessary, from the extreme base of the stem upwards to, and including, the peduncle. The nature, position, and course of the internal strands were thus studied in all parts of the axis; upper and lower, node and internode.

The general course and origin of the medullary strands, as seen in the lower region of the stem, and which is typical of the genus as a whole, is shown in Fig. 2, representing a median radial section.[1]

Stem 1.

Like nearly all plants of this order, the primary bundles composing the vascular ring are strongly individualized and more or less irregular in their alinement, as seen in transverse section. There is a wide pith which at certain levels becomes in part lacunar.

In the extreme base of the stem, at, or just below, the ground-level, and below that of the insertion of the lowest leaves, viz. immediately above where root-structure ends and a pith makes its appearance, two or three vascular bundles pass into the centre of the pith from the vascular ring. Of these bundles, one rejoins the ring at a slightly higher level, while the two others die out, i.e. end blindly *in situ*. Before one of them has died out, a few large bundles pass into the periphery of the pith from the ring, where they branch and anastomose and, at a rather higher level, give rise to a number of bundles in the pith-centre. Where all this occurs is the region of the lower crowded leaf-nodes. Higher up, these bundles constituting the central system die out *in situ*. But before they have all died out a new system of peripheral bundles arises close to the vascular ring; these bundles arise *de novo*, i.e. in the pith without any relation to the ring. At first consisting of phloem only, they soon acquire a considerable amount of xylem nearly all round the phloem. At a higher level some of the largest of these bundles join the ring. Slightly higher still, great numbers of tiny phloem-strands arise *de novo* in the central region of the pith and, a little higher up, the peripheral bundles above cited dwindle in size, and, together with the central small strands, form a single system of medullary strands throughout the pith. The smaller strands, consisting of phloem only, occur everywhere, even in the embouchement of the rays in the vascular ring. Tracing the structure still higher, the central strands and the smaller of those at the periphery decrease greatly in number, until only a few remain over in the central region; at the same time the larger peripheral strands give

[1] All the figures illustrating this paper are diagrammatically drawn. The parts shown black represent xylem, the dotted areas represent phloem.

rise by branching to a fresh system in that region, all of which are vascular bundles and are closely contiguous to the xylem of the ring-bundles.

The above-described structure obtains in the lower region of the stem.

At a node in a higher region of the stem where a branch is given off, two or three of the peripheral medullary bundles, occurring on the side on which the branch and the leaf-bundles are inserted, increase much in size from below upwards and become incompletely amphivasal in structure; they eventually anastomose with the bundles of the ring, and then pass outwards through the gap to form the adaxial portion of the vascular cylinder of the branch, becoming perfectly amphivasal before doing so.

The medullary strands of the stem pass up into the *peduncle*, in the lower part of which they consist of phloem only. As the lacuna makes its appearance the central strands die out *in situ*, so that in the higher part of the organ the strands, which have here nearly all acquired a little xylem on their *outer* side, are peripheral, one occurring opposite each bundle of the ring and forming with it the vascular supply of a floret.

Stem 2.

This was a thicker-stemmed individual than the last. In the swollen subterranean portion of the stem, at the level of insertion of the lowest leaves and where, as seen in transverse section, the leaf-traces are passing out, two or three vascular bundles arise *de novo* at the periphery of the pith. Here we see a difference from what obtained in the individual last described, where the first-appearing medullary strands arose from the vascular ring. Almost at the same time as these bundles arise *de novo* they form connexions with the vascular ring: this case may be thus regarded as a transitional one between those in which the bundles arise *de novo* and maintain for some distance an independent course and those in which the bundles arise from the vascular ring. A short distance below where these bundles arise the pith is seen to be full of short tracheides scattered promiscuously through the tissue.

The medullary bundles at once divide up and anastomose together and give off branches into the centre of the pith. The large peripheral ones appear to be directly connected with the leaf-traces, but are not direct continuations of these last, which is a very different thing. Rather smaller bundles occur here and there in the embouchement of the rays.

At a higher level, the bundles of the two medullary systems, viz. the large anastomosing peripheral, and the smaller, central, branching bundles, become entirely devoid of lignified xylem. At a still higher level, viz. at about the top of the swollen underground portion of the stem, the central strands die out *in situ* for the most part, but a few of them unite with the peripheral ones.

From a study of the medullary system of the stem in these two

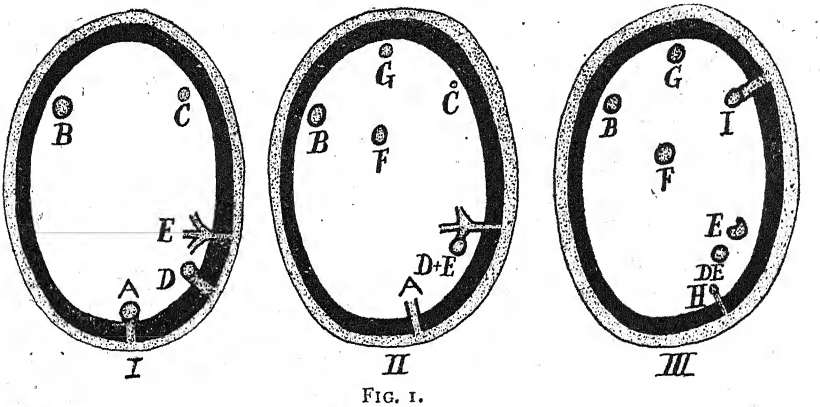


FIG. 1.

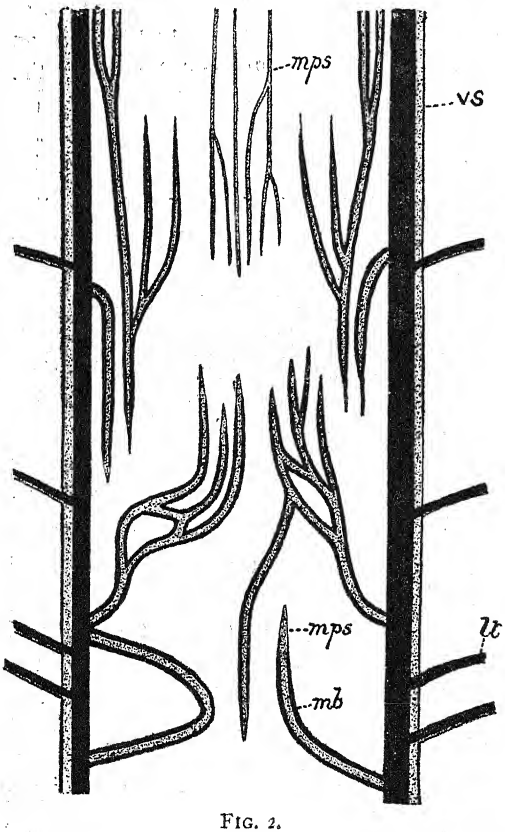


FIG. 2.

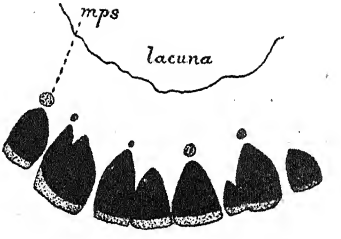


FIG. 3.

FIG. 4.

FIG. 1. *Tragopogon orientalis.* Transverse sections of the extreme base of the central cylinder of the stem, numbered from below upwards. The medullary strands are lettered alphabetically in the order of their appearance. (Diagrammatic.) FIG. 2. *Tragopogon.* Longitudinal section of the lower part of the central cylinder of the stem, showing the typical origin and course of the medullary strands in the genus. (Diagrammatic.) *lt* = leaf-trace; *mb* = medullary bundle; *mps* = medullary phloem-strand; *vs* = main vascular system (referred to in other diagrams as the 'vascular ring'). FIG. 3. *Tragopogon orientalis.* Segment of vascular ring, showing internal-phloem strands (*mps*). × 10. FIG. 4. *Tolpis barbata.* Vascular ring, showing transitions between some of its constituents and the medullary bundles (*mb*). Rudimentary medullary strands (bundles and phloem-strands) shown at *ms*. × 4.

individuals it can be seen that very considerable variation occurs in the lowest region of the organ as regards the origin of the bundles below and their history and behaviour on being traced upwards.[1] But the essential ground-plan of the structure is the same in both.

The structure of *T. orientalis*, L. (Figs. 1 and 3), and *T. porrifolius*, L., is essentially the same. In the latter plant the arc-shaped internal-phloem strands, of the higher part of the stem, each closely adpressed around the sclerotic sheath of a bundle of the vascular ring, and giving the bundle its 'bicollateral' character, pass upwards into the capitulum and go to form part of the normal phloem belonging to the bundles of the individual florets. But some of the medullary phloem-strands die out *in situ* before reaching the inflorescence.

Leaf.

The leaf in this genus is rather reduced and grass-like. Hence we should expect a corresponding condition of the vascular system. This consists of a curved row or arc of alternately larger and smaller bundles. In *T. pratensis* internal-phloem strands are completely absent, at least in all leaves examined by the writer.

In *T. orientalis* internal-phloem strands occur throughout the typical portion of the stalk of the radical leaf on the ventral side of the five larger median bundles of the row, but are absent from the smaller lateral bundles. One to three strands occur opposite each bundle. The internal-phloem strands die out completely *in situ* in the basal sheathing portion of the leaf, so that in the extreme base of the leaf they are absent. They also die out above in the midrib of the lamina.

In *T. porrifolius* the three or four median bundles of the row, about half an inch or more above the base, have each several small strands of internal phloem arranged in an arc either closely contiguous to the bundle or separated therefrom by two or three layers of cells; the lateral bundles are devoid of them. The internal-phloem strands die out below, and the bundles in the leaf-base are entirely without them.

Hence it will be seen that the internal-phloem strands of the stem in this genus have no connexion whatever with those of the leaf, though they are doubtless entirely homologous, and, in the writer's opinion, will have been ancestrally directly continuous, therewith.

Scorzonera.

Stem.

This plant is very closely allied to *Tragopogon*. In conformity herewith the character of the medullary bundle-system is extremely similar. The same individual variations obtain as in that genus. For example, in

[1] In the higher aerial internodes and in the peduncle the structure of both stems is almost identical. In the stem-base of other species a strand may arise *de novo* in the *centre* of the pith.

one specimen of *S. hispanica*, L., all the medullary strands, when traced downwards, die out *in situ* in the extreme base of the stem, while in another specimen they all pass into the vascular ring. In one individual the medullary strands are complete vascular bundles throughout the axis of the plant, in another vascular bundles and phloem-strands occur together. A mode of origin of the medullary strands was observed in this plant which was not described for *Tragopogon*.[1] In *S. hispanica* the vascular ring of a vegetative branch was traced downwards to its complete union with that of the main stem. As this took place the medullary strands of the branch pass into the pith of the stem, fusing up there to form a few large strands. Also a bundle from the middle of the abaxial portion of the vascular ring of the branch passes into the same region of the pith of the stem, its place in the branch-ring being filled by the incoming median leaf-trace bundle. Where the incoming branch is a *peduncle*, most of the inverted medullary bundles, occurring in juxtaposition to the bundles of the ring, do not change their position as these last pass inwards to form part of the vascular ring of the stem. Both in this species and in *S. purpurea*, L., minute phloem-strands may arise, either from the vascular ring at the point of junction of the two axes, or *de novo*, and form a rudimentary central medullary system of the branch. In *S. purpurea* it was noted that the slenderer branches of the stem have no medullary strands, but at their nodes a medullary bundle may appear in the bay caused by the exit of the cylinder of a secondary branch.

In a fairly strong leafy lateral peduncle medullary bundles occur in the first two or three internodes, but completely die out above.

Leaf.

In *S. hispanica* there is the same structure as in *Tragopogon*, with internal-phloem strands which die out below. But on the adaxial side of the pith there are a few small bundles, devoid of internal phloem, each situated at a different radial distance from the leaf-centre, in a portion of tissue projecting into the large lacuna. These bundles probably represent a remnant of the ventral portion of the erstwhile existing vascular ring of the leaf, and are an indication that this organ, which has the same external conformation as in *Tragopogon*, has been reduced in size.

In *S. purpurea* the internal phloem behaves in the same way. But it was noted, in the case of one node in the stem, that while the median bundle of the dorsal row of the leaf was traversing the cortex three minute internal-phloem strands arose *de novo* on its ventral side; immediately before the bundle passes into the vascular ring of the stem the internal-phloem strands unite with the ordinary external phloem of the stem.

[1] Essentially the same thing was, however, observed in *T. pratensis*.

Podospermum laciniatum, DC.

Stem.

Herbarium material only.

Very numerous small phloem-strands occur in the pith, aggregated towards the centre; none occur at the periphery.

P. calcitrapifolium, Boiss.

Stem.

Herbarium material only.

No medullary bundles or phloem-strands were found.

Scolymus.

Stem.

This genus possesses numerous small phloem-strands at the periphery of the pith, which are continuous into the branches. Opposite the leaf-trace bundles they are in the form of vascular bundles of much larger size, which unite with the vascular ring after the leaf-bundles 'exit'. The medullary phloem-strands, if traced downwards into the base of the stem, become fewer in number, and, for the most part, well-constituted amphivasal bundles. At the lowest level, i.e. immediately below the entrance of the lowest leaf-traces, they die out *in situ*. The phloem-strands (a bundle here and there amongst them) persist into the highest part of the stem and peduncle, and go to form part of the supply system of the capitulum.

S. maculatus, L., was the species examined specially for the course of the strands. *S. hispanicus*, L., has essentially the same structure.

Leaf.

There is a simple arc of large, widely-separated bundles. There is no trace of intraxylary phloem in any part of the petiole or midrib.

This is, therefore, one of the rather rare cases in which the leaf exhibits a more advanced, i.e. reduced, structure than the stem.

Picridium tingitanum, Desf.

Stem.

Kruch found medullary strands in this plant. The present writer found that the three specimens which he examined from the Herbaceous Ground at Kew were entirely devoid of them, save for the invagination into the pith of two amphivasal bundles in the lower end of one of the branches.

Leaf.

There is a simple arc of large bundles alternating with very small vestigial ones.

Tolpis barbata, Gaertn.

Stem.

There are two kinds of medullary bundles occurring as an invariable feature of this plant. Minute bundles and phloem-strands arise *de novo* in each internode opposite the bundle of the ring which eventually passes out as the *median* bundle of the leaf-system; they form an arc around its xylem (Fig. 4, *ms*). After the leaf-bundle has passed out, they unite with the ring on each side of the gap.

At each node, at the mouth of the bay formed by an outgoing branch, similar medullary strands arise *de novo* and, after the exit of the branch, die out *in situ*, though one or two may join the ring of the stem.

In another stem, in one of the shortest, lowest internodes, eight or ten small, variously-orientated, amphivasal bundles arise *de novo* all round the periphery of the pith; in succeeding internodes above they gradually die out.

Here also the same small medullary strands arise opposite the outgoing median leaf-bundle.

Besides the above-mentioned medullary strands, another set, of quite different origin, sometimes occur in certain internodes. These are well-developed bundles of the ring which pass into the pith and become amphivasal (Fig. 4, *mb*). More often, they are seen as constituent parts of the ring projecting into the pith. In fact, this plant is one of the best for showing how the vascular ring of the stem of Compositae has been derived from an original scattered system of bundles.

Leaf.

As the three main leaf-bundles pass outwards into the cortex they each acquire on their ventral (inner) side a number of small inverted bundles; these are derived from the stem-ring; they persist upwards into the leaf, and represent the peripheral medullary system of strands of that organ.

Lactuca virosa, L.

Stem.

Medullary strands occur throughout the stem. In the higher part of this organ phloem-strands only occur (Fig. 6). In the basal region they acquire xylem and become bundles (Fig. 5). In this basal region, at a still lower level, they lose the xylem, and eventually either die out *in situ* or join the vascular ring. The amount of xylem in the medullary strands varies with the thickness of the stem, and is always secondary in origin. The fate of the strands, if traced upwards, is fourfold. Some of them end blindly in the pith. Some pass into the vascular ring. Others pass out to form the medullary system of a branch; but in one and the same plant

the branch-system may arise both in this way and also *de novo* in its pith-tissue. The small peripheral phloem-strands persisting in the higher part of the stem and peduncle become part of the ordinary vascular system of the flowers.

The above structure is also exhibited by *L. Scariola*, L., *L. saligna*, L., and *L. sativa*, L.

In *L.* (*Mulgedium*) *alpina*, Bth. and HK., *L. Plumieri*, Gren. and Godr., *L. Bourgaei*, *L. macrantha*, C. B. Cl., and *L. perennis*, L., the medullary

FIG. 5. *Lactuca virosa.* Segment of vascular ring from lower part of stem; in the pith are scattered bundles and phloem-strands. × 4.

FIG. 6. *L. virosa.* Ditto from upper part of stem, showing arc-shaped internal-phloem strands. × 4.

strands (both bundles or of phloem only) are few in number or completely undifferentiated and vestigial. In some species they only occur at a certain level of the stem, in others solely at the nodes.

In *L. hastata*, DC., and *L. macrophylla*, A. Gray, medullary strands are completely absent.

Leaf.

In *L. alpina* and *L. Plumieri* there is a complete vascular ring. A medullary system of very small scattered bundles and phloem-strands occurs close round the central lacuna, and also a peripheral system of fewer

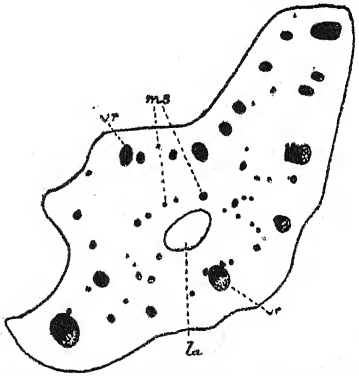


FIG. 7. *Lactuca Plumieri.* Petiole, showing vascular ring (*vr*) and medullary strands (*ms*); *la*, lacuna. × 4.

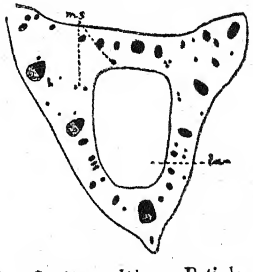


FIG. 8. *Lactuca alpina.* Petiole, showing vascular ring and medullary strands (*ms*); *la*, lacuna. × 4.

strands (Figs. 7 and 8). The medullary system ends blindly in the tissue below, as do also the smallest bundles of the ring. This structure is also shown by one or two other species.

In the leaf of *L. virosa* there is a simple dorsal arc of bundles; but vestiges, in the form of minute bundles, of the adaxial portion may occur. The medullary system consists of small phloem-strands occurring on the immediate inner side of the dorsal bundles; they arise both from these latter and *de novo* in the pith.

In *L. Scariola* medullary phloem has vanished from the leaf. In *L. macrophylla* the typical region of the petiole (about half-way up) has a complete vascular ring, some of the adaxial bundles of which are very minute and end blindly *below*, while the rest unite with those on the dorsal (abaxial) side. The same thing happens in the *upper* region of the petiole. Thus, we see in one and the same organ the evolution of the more recent simple arc of bundles (in other species characteristic of the entire petiole) from the more primitive ring (characteristic of the entire petiole of still other species). In one section a minute rudimentary medullary strand was observed. In those species in whose stems medullary strands are either absent or almost absent, they often occur, in the form of small phloem-strands, on the ventral side of the leaf-bundles in the cortex in or near a node. It is important to note that that segment of the stem-cortex in which bundles occur which have passed in from a leaf, the place of insertion of which latter may be a considerable vertical distance above, must be regarded as constituting part of the leaf, viz. its decurrent base. Further, the small phloem-strands occurring on the ventral (inner) side of leaf-bundles in this region are to be regarded as belonging to the peripheral medullary system of the leaf. In some species, as in *L. perennis*, these small phloem-strands occur on the inner side of the median leaf-trace bundle *while it still forms part of the stem-cylinder*; they end blindly in the pith-tissue if traced downwards. These strands in that species do not pass out into the cortex with the median leaf-bundle, but they fill up the gap in the outer portion of the vascular ring of the axillary branch caused by the median leaf-bundle passing outwards from it. Much the same sort of thing was observed in *L. macrantha*, but, at least in one node, the median leaf-bundle was devoid of internal phloem at every stage. In all these species the *lateral* leaf-bundles also have internal-phloem strands which are derived from the ordinary external phloem of the stem-ring.[1] Before the bundles pass out into the free petiole the internal phloem fuses completely with the external phloem of the bundle.

This nodal region of the stem, where the leaf-bundles are passing in towards the ring, is a conservative region where ancestral characters would be likely to persist.

The presence of phloem-strands on the ventral side of the median leaf-bundle while it still constitutes part of the stem-ring is a fact of the

[1] In *L. Bourgaei* it was noted that the more lateral of the internal-phloem strands of the *median* leaf-bundle have the same origin.

first importance, for two chief reasons: in these species these phloem-strands represent the last vestigial remnant of medullary bundles in the stem, and it is of paramount interest that they occur in connexion with the main or median leaf-bundle; as the phloem-strands are in all respects perfectly similar to those which occur on the ventral side of the leaf-bundles in the cortex (i. e. in the decurrent leaf-base), the two sets must be regarded as homologous; it follows, further, from this that they must be regarded as homologous with the phloem-strands (or bundles) occurring on the ventral side of the *bundles of the petiole* of other species. The final obvious deduction is that the small ventral strands of the bundles of the petiole, not only of this genus but of all genera in which they occur, *represent a peripheral medullary bundle-system* of the petiole which is homologous with that of the stem. And the point of chief interest is that, *where they occur in the leaves of those plants whose stems show no trace of medullary strands, they indicate that medullary strands were once a constant feature of those stems.*

Even were the above deductions unconvincing, there is no other possible explanation of these small ventral strands of the leaf.

The medullary phloem-strands or bundles, whether peripheral ('internal phloem') or central, of the free petiole arise, as regards their immediate origin, quite independently, and have no connexion with the internal-phloem strands of the 'cortex' of the stem above described. But this fact does not interfere with the conclusion that the two sets are completely homologous with each other. In a case like that of *L. perennis* medullary strands are absent in the free petiole, but we see them beginning to be abstricted off from the external phloem of the larger bundles. In the decurrent base of the leaf ('stem-cortex') they have been completely abstricted off and occur on the ventral side of the bundles.

Sonchus.

This genus is closely allied to *Lactuca*. There is space only for a brief mention of the structure.

S. asper, Hill.

Stem.

The medullary phloem-strands constitute a fairly independent system; they form connexions at the node with the bundles of the vascular ring of both stem and branch.

The most median of the internal-phloem strands of each leaf-bundle enter the pith and form there an independent strand, but the more lateral ones unite with the external phloem of the stem-cylinder. The former of these two facts is of great interest in connexion with what was observed in the case of *Lactuca*.

S. arvensis, L.

Stem.

There are medullary phloem-strands at the periphery of the pith, and at the nodes they are also scattered throughout the pith.

Leaf.

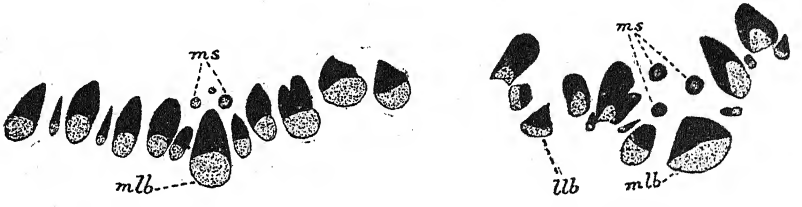
An arc of bundles. The median one has two small internal-phloem strands; these die out just before the bundle enters the stem.

Crepis.

C. biennis, L.

Stem.

Considerable variation in the development of medullary strands was found in this species. In cultivated specimens traces of them were found. In wild specimens no trace of them could be discovered. In those cases in which they occur it is in the lower portion of the stem, the higher being devoid of them.[1] Moreover, they are always found at, or in the near neighbourhood of, a node. They arise both *de novo* and from the vascular



FIGS. 9, 10. *Crepis biennis*. Segments of vascular ring of stem, showing medullary strands (*ms*) in the bay formed by the outgoing median leaf-bundle (*mlb*). One of the lateral leaf-bundles at *llb*. × 9.

ring, as seen in tracing them from below upwards, and they branch and anastomose, as is usual in other genera. They occur, either as vascular bundles or phloem-strands, always opposite and contiguous to a leaf- and branch-trace (Figs. 9 and 10). Most of them pass out into either a branch- or leaf-trace to constitute part of the normal vascular tissue, but others were seen to join the cylinder of the stem. The fate of one strand was interesting. It arose *de novo* in the *outer* part of the bay formed by an outgoing branch-cylinder. It first of all branched into two; each strand so arising formed connexions with the branch-cylinder on either side. They then passed out, after one of them had again divided into two, with the leaf-trace bundle, while the branch-cylinder closed up to the inside of

[1] In another cultivated specimen, from a distinct batch of seedlings, one or two nodes in the upper part of the stem exhibited each a well-developed medullary amphivasal bundle.

12 DEC 1913

them. The three strands, consisting of phloem only, formed, subsequently, connexions with the phloem of the branch-cylinder, and eventually they constituted the internal-phloem group of the median leaf-bundle in the cortex.

The lateral leaf-bundles in the cortex possess internal-phloem strands which are derived from the normal phloem of the stem-cylinder.

In *C. virens*, L., medullary strands occur in some individuals, of both wild and cultivated specimens, in fair numbers; in other individuals they are quite absent.

In *C. taraxacifolia*, Thuil., a cultivated specimen showed a single sub-amphivasal, medullary bundle at one node in the bay formed by an outgoing leaf-bundle; at a lower level it joined the cylinder. Two wild specimens showed no trace of medullary strands.

In *C. nicaeensis*, Balb., and *C. sibirica*, L., medullary strands are quite absent from the stem.

Leaf.

The following description relates to *C. taraxacifolia*:

The vascular structure of the petiole in this species is of a rather more ancestral type than that of the other four, with which, otherwise, it generally conforms. That of *C. biennis* is shown in Fig. 11.

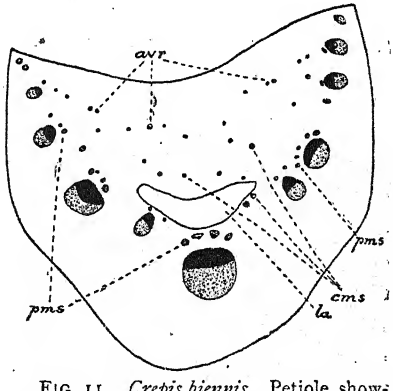


FIG. 11. *Crepis biennis*. Petiole, showing the vestigial character of the adaxial portion of the vascular ring (*avr*), the central medullary strands (*cms*), and the peripheral medullary strands (*pms*); *la*, the lacuna. × 12.

At the extreme base is the usual simple arc of bundles. Higher up, in the typical region, a row of minute bundles with inverse orientation (xylem towards the centre) occurs, representing the ventral or adaxial portion of the vascular ring. Between this adaxial row and the abaxial one are scattered minute phloem-strands, with here and there a bundle; these represent the medullary system of strands. There is a small central lacuna.

All these minute bundles and phloem-strands, i. e. the adaxial portion of the ring and medullary system, arise *de novo* below; in other words, end blindly if traced downwards. If traced upwards they also end blindly at about the level where the petiole merges into the midrib.

The large median bundle of the dorsal or abaxial row is more or less arched and abstricts off on either side a small bundle or phloem-strand, with inverted orientation, on to its ventral side.

The smaller strands of the abaxial row, alternating with the larger ones, are situated at a slightly shorter radial distance from the lacuna than the larger bundles; this is important as indicating that they represent a transitional series between the central medullary strands and the larger bundles of the abaxial row..

The vascular structure of this genus is instructive. We have seen that medullary strands occur in the stem of some species, but not in that of others; and in the stem of certain individuals of the same species, but are absent in that of other individuals. Moreover, in the latter cases they may occur in certain internodes of the same stem but are absent in others. These facts clearly point to the conclusion that the medullary strands are vestigial (i. e. ancestral) structures which are on the verge of extinction in the stem.

On the other hand, the petioles of *all* species examined showed the very obvious presence of medullary strands in larger or smaller numbers; but they are always of extremely small size and on the verge of extinction, hence no longer of any use to the plant. They represent an ancestral feature which once prevailed in both stem and leaf of all species.

Picris hieracioides, L.

Both wild and cultivated specimens were investigated.

Stem 1.

Medullary strands are absent from the internodes and from the branchless nodes. But in the nodes at which branches are given off they occur, as in the stem of *Crepis biennis*, to which genus *Picris* is closely allied.

At one node a bundle passed into the stem-ring from the vascular ring of the branch and took up a position in the inner entrance of the branch-bay, where it divided into two; these two bundles then gradually died out at a higher level in the node as the stem-ring closed up behind the outgoing branch-ring. At another node two medullary bundles arose *de novo* at the bay-entrance, fused together, and died out *in situ* while the ring closed up. At another node two bundles arose *de novo*, one of which divided; of these three one had apparently died out in the next section; the remaining two also died out before the ring had closed up. At two other nodes the same events occurred. All the above-mentioned nodes are at successively higher levels on the stem.

Stem 2.

At all the nodes in the lower part of the axis where no axillary branches, but only small buds, occur no medullary strands are to be found. In the lowest of the branch-nodes, however, medullary strands occur and

behave as in stem 1. But in all higher branch-nodes no medullary strands exist at all.

A feature of the stem of this species is the very narrow cortex, the wide pith, and the existence of minute bundles (sometimes reduced to phloem only) between, and slightly farther to the outside of, the large bundles of the ring (Fig. 12). These rudimentary bundles, which can hardly represent an adaptive, but rather a vestigial character, and their relative position furnish evidence of the primitive scattered arrangement of the bundles of the ring in this plant, it being merely a rather good example of what has happened in the stems of most other Compositae, viz. a pushing outwards of the larger, innermost bundles towards the periphery, whereby the outer smaller ones inevitably become reduced and on the way to extinction owing to lack of adequate space.

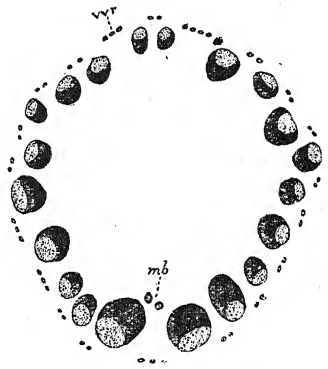


FIG. 12. *Picris hieracioides.* Vascular ring of stem, showing its vestigial outermost constituents (*vvr*) and two peripheral medullary bundles (*mb*). × 9. (In this figure the small vestigial strands have been placed slightly farther to the outside than they are in the actual specimen.)

The xylem of all the bundles in the nodal region is very V-shaped (Fig. 12); this means that each of these bundles pertains partly to the pith and partly to the vascular ring, hence their semi-amphivasal structure; in other words, that they are the constituents, in this conservative region, of an original scattered system of bundles. This is but typical of what occurs in most Compositae (*Tolpis barbata and Picris strigosa* are merely cases of this in more primitive form).

P. strigosa, Bieb.

Stem.

Herbarium material only was available of this species. The vascular ring, as seen in transverse section, consists of bundles with rather V-shaped xylem, with very numerous tiny ones between them at the same level.

The medullary system of bundles is very striking and pronounced, consisting of a ring of large, perfectly circular amphivasal bundles with protoxylem in varying positions (Fig. 13). They occur at the periphery of the pith, and are connected with the ring by two or three bundles of an intermediate series. This species, therefore, shows the more primitive structure of the two.

Leaf.

In the typical part of the rachis (there is no petiole) of *P. hieracioides* there is an arc of alternately large and small bundles, some of the latter

being reduced to phloem. As in the case of *Crepis*, the median bundle abstricts off a small phloem-strand on either side. There are no central medullary bundles or phloem-strands. But the small bundles or phloem-strands alternating with the larger bundles of the arc really represent the outermost series of a medullary system, as shown by their situation slightly nearer the ventral side than the large bundles and also close to the edge of the lacuna. But these last, again, represent the outermost (dorsal) of the whole system of scattered bundles which once existed in the leaf of all Compositae. These remarks also apply to the structure of the petiole of *Crepis* and *Lactuca*.

Helminthia echioides, Gaertn.

Stem 1 (Wild).

The characteristic feature of this plant is the large number of very small phloem-strands which are scattered uniformly throughout the pith (Fig. 14). No instance was seen of the occurrence of any xylem in these strands, and they are of very uniform size. A few arise *de novo* in the pith

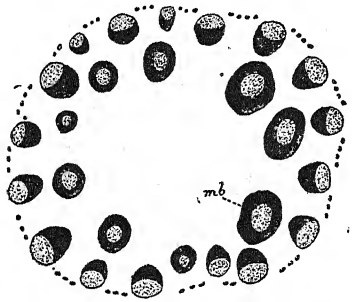


FIG. 13. *Picris strigosa*. Vascular ring and medullary bundles (*mb*). × 10.

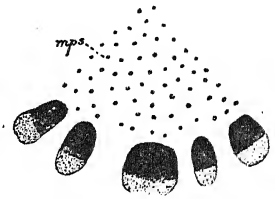


FIG. 14. *Helminthia echioides*. Segment of cylinder of stem, showing vascular ring and the scattered medullary phloem-strands (*mps*). × 12.

of the extreme thickened base of the stem, where they branch up rapidly to form the large number seen at a higher level in the thinner aerial portion. They have connexions with the phloem of the ring through the leaf-trace rays.

The medullary phloem-strands are entirely devoid of hard bast.

At the node, in the bay formed by an outgoing branch, the medullary strands increase in size; some of them here join the phloem of the stem-ring, others pass out and become the medullary strands of the branch. Here we see that where a medullary system is well developed in the stem it is continuous with a similarly well-developed system in the branch.

In three cultivated stems, which are more robust than those of the wild plant, the structure is precisely the same throughout.

In the *peduncle* the medullary phloem-strands are very minute and rudimentary and sometimes completely absent.

Leaf.

In the midrib the scattered arrangement of the bundles is very evident. The largest bundle is situated by itself on the dorsal side. Farther to the ventral side is an arc of fairly large bundles, the terminal ones of the arc being at the inner corners of the midrib. Unevenly distributed in the remainder of the space to the inner side of the arc are eight or nine very much smaller *bundles* of varying sizes, three or four of which on the extreme ventral (adaxial) side are very rudimentary and almost extinct. The small medullary bundles are orientated like those of the arc. We thus see that the medullary strands of the leaf are better developed and less reduced than those of the stem, the latter having completely lost their xylem, while the former have retained it. This is another piece of evidence in favour of the leaf-structure representing the ancestral type. The very rudimentary strands on the extreme ventral side of the midrib may represent the reduced adaxial portion of the original cylinder.

Taraxacum officinale, Weber.

Peduncle.

The vascular ring consists of three sizes of bundles, all practically in a line, representing, as in so many of such cases, the original scattered triserial arrangement which existed before the advent of the wide lacuna of the pith.

Leaf.

The above-stated view, that in the stem (or peduncle) of this plant, and of all Cichoriaceae, there was an aboriginal scattered system of bundles, is confirmed by the structure of the leaf. Here, around the central lacuna, are a large number of very scattered strands of which only a few (3–5) near the edge of the lacuna on the dorsal side are well-developed bundles; lying between and to the outside of these last are considerable numbers of widely-distributed and, in many cases, exceedingly small and rudimentary phloem-strands of obviously vestigial nature.

CYNAROIDEAE.

Cynara Cardunculus, L.

Stem.

This is one of the Compositae in which the scattered arrangement of the bundles is most pronounced. The vascular ring consists of this complex system, many of the bundles being inversely orientated, others orientated sideways. They are of all sizes: some extremely small, others reduced to mere rudiments and fragments. One or two of the large inverted bundles

are seen to have an arc of three or four small normally-oriented bundles around their xylem. Such curious groups of concentrically-arranged bundles are what one so frequently encounters in the leaves of this order; it is exceedingly rare to find such bundle-groups passing in, as in this case, to constitute part of the *stem*-ring; it must be regarded as one of the primitive features of this last.

The great radial thickness of the ring, consisting of scattered bundles, the very diverse orientation of the latter, their great variation in size, the presence of numerous rudimentary bundles intercalated amongst the others, must all be regarded as primitive features, for none of them can be solely explained on the grounds of adaptation to the very large capitulum with its great multitude of florets. For there is clearly no reason why the bundles should be scattered instead of being ranged in line, nor why they should be diversely orientated, or of different sizes and degrees of development. It is simply that the considerable thickness of stem in this plant affords room and play for the original primitive structure of the cylinder; hence this has been retained.

C. Scolymus, L.

Peduncle.

All the features described above for the stem of that species are present, especially in the upper part of the peduncle of this species, in a greatly accentuated form. The structure probably represents the most extreme

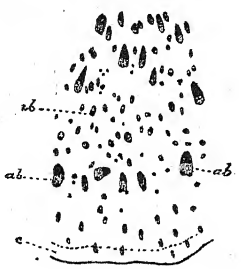


FIG. 15.

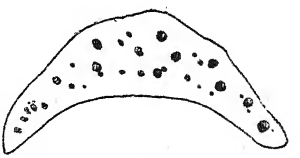


FIG. 16.

FIG. 15. *Cynara Scolymus.* Segment of vascular cylinder and cortex (*c*) of uppermost portion of peduncle, showing the scattered disposition of the bundles. Some of these are inversely orientated (*ib*); others are partially amphivasal (*ab*); others lie sideways. There is here no distinction between medullary strands and those of the ring. × 4. FIG. 16. *Cynara Cardunculus.* Petiole, showing scattered disposition of the bundles. × 4.

case in the Compositae of the primitive scattered disposition of the bundles composing the vascular ring (Fig. 15). The radial thickness of the ring here equals the diameter of the pith. The majority of the bundles are much smaller than those of the stem of the last species, and there are a larger number of rudimentary strands. A few of the innermost bundles are completely amphivasal, which is another primitive feature to be added

to those already enumerated. Most of these bundles represent traces from the large involucral bracts. There is no discernible limit between stele and cortex.

Leaf.

That the extremely marked scattered disposition of the bundles in the stem and peduncle has no direct connexion, as regards origin, with the supply of the numerous florets in the capitulum is shown by the fact that this same scattered arrangement occurs in pronounced form in the petiole of the leaf (Fig. 16). The bundles are less numerous relatively to the available space, but exhibit the same differences in size and orientation, and are also here and there in a rudimentary condition. None of these features can be represented as in any sense adaptive to the needs of the plant; it therefore follows that they are primitive and ancestral. This conclusion thus applies to the structure of both leaf and stem. According to the view which is set forth throughout this paper, the leaf has imposed its structure on the stem, not vice versa.

Centaurea.

Stem.

In *C. macrocephala*, Puschk., there is a very large capitulum; this implies a stout roomy stem and peduncle to support and feed it. Hence this allows space for the primitive scattered disposition of the bundles to reappear, which is, in fact, what we find in this species. All the features, with the exception of amphivasal bundles, described above for *Cynara* occur here, but on a very reduced scale.

In all other species the bundles are almost in line, though the ring betrays in every case its derivation from a scattered system of bundles.

ARCTOTIDEAE.

Gundelia Tournefortii, L.

Stem.

Herbarium material only was investigated.

Small phloem-strands occur scattered, at wide distances apart, in the pith (Fig. 17).

SENECIONIDAE.

Senecio Petasites, DC.

Stem.

At the periphery of the pith and opposite, and exactly median to, the arc of leaf-bundles in the cortex which has recently left the vascular ring, occur five or six rudimentary medullary strands, one or two being attached

to the sclerotic tissue covering the xylem of the ring-bundles. This is the only case in which medullary strands have been observed in this tribe.

S. clivorum, Maxim.

Leaf.

The petiole contains a large number of variously orientated and sized bundles scattered indiscriminately throughout the tissue (Fig. 18). The petiole of *S. Petasites* does not show this scattered disposition, but a more reduced type of structure which has evidently been derived therefrom, and is much more primitive than that of the stem.

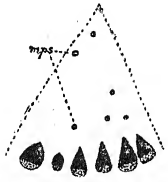


FIG. 17. *Gundelia Tournefortii.* Segment of cylinder of stem, showing scattered medullary phloem-strands (*mps*). × 4.

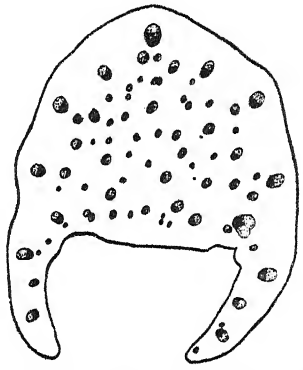


FIG. 18. *Senecio clivorum.* Petiole, showing extreme scattered disposition of the bundles. × 5.

Petasites officinalis, Moench.

Peduncle (Male Plant).

The scattered disposition of the bundles composing the main vascular system is very pronounced, especially in the upper part of the organ (Fig. 19). The presence of small, rudimentary, and abnormally orientated strands suggests the derivation from an even more pronouncedly 'Monocotyledonous' type of structure.

Petiole.

This organ exhibits one of the best examples known of the primitive scattered distribution of the bundles, this feature being directly connected with the large size of the leaf, adequate space being present for the preservation of the original structure. There is a complete vascular ring with great numbers of bundles of varying size and orientation, some of which are rudimentary, distributed throughout the ground-tissue (Fig. 20). In the basal region, where the central lacuna has greatly dwindled in area, further strands of rudimentary structure appear nearer the centre.

It would be difficult to attribute an adaptive significance to the vascular structure of this organ; it must, therefore, be aboriginal.

HELIANTHOIDEAE.

Rudbeckia maxima, Nutt.

Stem and Peduncle.

There are two well-marked features of the vascular ring which strongly suggest its derivation from a primitive scattered system of bundles, viz. (1) the extreme irregularity of alinement of its component bundles, which are at very variable radial distances from the centre of the pith, and (2)

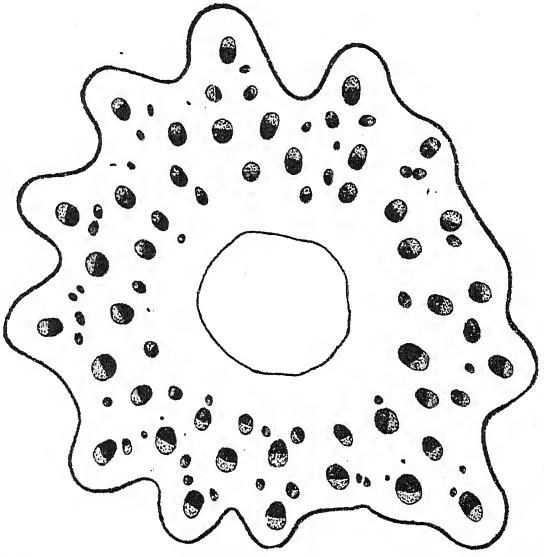


FIG. 19. *Petasites officinalis.* Peduncle of male plant, showing scattered disposition of bundles. × 5.

the great difference in the size of the bundles, some being very small and even rudimentary, and irregularly orientated.

There are very well developed medullary bundles, of perfect amphivasal structure, forming a ring about half-way between the ring and the central lacuna. Nearly all are seen to be branching into two or three (Fig. 22). In the peduncle some of the medullary bundles occur nearer the ring, and some are extremely rudimentary.

If traced downwards the medullary bundles are all seen to die out *in situ* in the region of the primary node, just above where root-structure begins. If traced upwards they, in some plants, disappear in the peduncle, either dying out *in situ* or passing into the ring. In other plants they persist into the receptacle of the capitulum, the same fate befalling them there.[1]

[1] In the uppermost region of the peduncle, and also in the receptacle, the lacuna has disappeared and, as a consequence thereof, medullary bundles occur in the centre of the pith.

It is an interesting fact, by no means confined to this genus, that in the receptacle or axis of the capitulum the bundles of the ring become completely amphivasal like those of the pith in the peduncle and stem. This the writer regards as the retention of a primitive feature, there having been no call for its modification in this part of the plant-axis as there has been in the case of the vascular ring of peduncle and stem. It cannot be explained as a necessary structure for the supply of the florets, but has been retained through not being adversely adapted to this purpose.

Leaf.

In the petiole and midrib there is a simple, regular arc of alternately large and small (some very minute) bundles (Fig. 23). Apart from the great disparity in size of the bundles, which is a primitive character, the structure is an advanced (reduced) one as compared with that of the stem, the bundles of the arc being regularly alined and there being a complete absence of medullary strands.

R. digitata, Mill. (*R. laciniata*, L.).

Stem and Peduncle.

The vascular ring shows the same features as in the last species. The cortex is extremely narrow. Medullary bundles are completely absent, although the lacuna is not more extensive than it is in the last species (Fig. 21).

Receptacle of Capitulum.

The vascular ring consists of three series of bundles of which the structure is amphivasal. The innermost are the largest. There are no central medullary bundles.

Leaf.

The structure is the same as in the last species.

In *R. californica*, A. Gray, *R. occidentalis*, Nutt., *R. Newmani*, Loud., *R. columnaris*, Sims, *R. pedunculari*s, Torr. and Gray, and others, medullary bundles are completely absent, and the leaf has a simple arc of bundles.

Echinacea purpurea, Moench.

Stem.

In the higher part of the stem there occur in the centre of the pith six or seven small bundles of amphivasal structure, consisting of soft bast entirely enclosed by xylem-fibres; one or two small vessels or tracheides were noticed in one bundle; one or two bundles are quite rudimentary, consisting of xylem-fibres only (Fig. 24). Owing to lack of material the

fate of these medullary bundles in the lower region of the stem was not ascertained. One or two internodes higher up they have increased in number by division and are thus smaller, and for some distance upwards

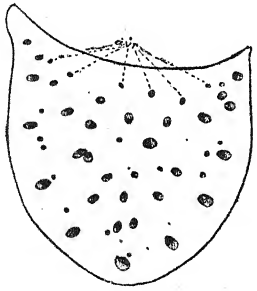


FIG. 20.

FIG. 21.

FIG. 23.

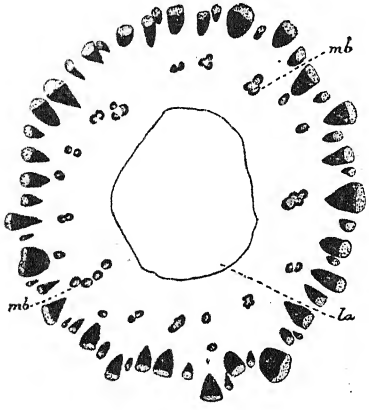


FIG. 22.

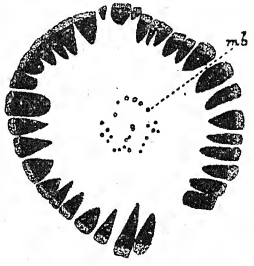


FIG. 24.

FIG. 20. *Petasites officinalis.* Petiole, showing well-developed medullary system of bundles. Bundles of adaxial portion of vascular ring at *avr.* × 4. FIG. 21. *Rudbeckia laciniata.* Vascular cylinder of stem, showing absence of medullary strands. × 4. FIG. 22. *Rudbeckia maxima.* Ditto, showing presence of medullary bundles (*mb*); *la* = lacuna. × 4. FIG. 23. *Rudbeckia maxima.* Leaf, showing complete absence of medullary bundles. × 4. FIG. 24. *Echinacea* (*Rudbeckia*) *purpurea.* Vascular cylinder of stem, showing rudimentary central medullary bundles (*mb*). × 4.

they traverse the nodes without any change in position or number. In the higher internodes they gradually dwindle in numbers as a result of fusion and of dying out *in situ* until, immediately below the second leafy node from the capitulum, they completely vanish.

The lateral branches are quite devoid of medullary bundles. In the peduncle small vestigial bundles or phloem-strands occur on the ventral side of the bundle which leaves the vascular ring to supply a floret; they arise *de novo* either in the sclerenchyma composing the ventral side of the bundle or free in the ground-tissue.

A smaller specimen examined showed no medullary bundles in the internodes, unless the two or three small insignificant strands of sclerenchyma opposite a few of the ring-bundles represent their vestiges. In the nodes, at each corner of the bay formed by the outgoing vascular ring of a branch, a bundle enters the pith and becomes amphivasal, only to re-enter the ring at a higher level just before the branch leaves the stem.

Leaf.

The scattered disposition of the bundles is very obvious in the petiole. In one specimen are two distinct series, of which the innermost consists of very small bundles, evidently vestigial and of no particular or useful function. In another specimen there are one or two minute, normally-orientated bundles opposite to, and within, the larger ones; one of the larger inner bundles is almost amphivasal, while other smaller ones have oblique orientation. In another specimen there are three distinct series of bundles, of which the innermost consists of numbers of very small, normally-orientated bundles, some of which end blindly below in the region where the bundles fuse up to form the simple arc which eventually passes into the stem; some also are seen to end blindly if traced upwards (Fig. 25).

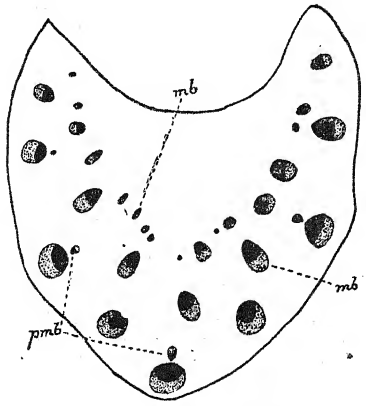


FIG. 25. *Echinacea* (*Rudbeckia*) *purpurea*. Petiole, showing medullary bundles (*mb*). The most peripheral of these (*pmb*) are recent lateral derivatives from the arc-bundles to which they are opposed. × 4.

Some of the outermost (dorsal) bundles have on their ventral side, and also at their inner corners, one, two, or three very small, inversely-orientated bundles, which, in the base of the petiole, fuse laterally with the large bundle (Fig. 25). One or two of these outer bundles are quite concentric, with central xylem, due to the fact that the small bundles, which in those just described are detached from the sides and become distinct strands on the ventral side, are in these cases never detached, but remain a constituent part of the whole bundle.

Dahlia excelsa, Bth.

Peduncle.

Herbarium material only was investigated.

There is a wide lacuna. In the pith, at wide distances apart, are a few more or less rudimentary concentric strands consisting, in some cases, of phloem only with cambial cells all round it; in other cases, in addition,

of protoxylem and one or two secondary vessels on the *outer* side. These medullary strands are very variable in size, some being very small.

D. Merckii, Lehm.

Stem.

The bundles of the ring have very V-shaped xylem, are very variable in size, and thus suggest their original derivation from a scattered disposition.

D. sp.

Leaf.

In the petiole is an incomplete ring of separate bundles and a complete absence of medullary strands.

Hemizonia corymbosa, Torr. and Gray.

Stem.

Medullary bundles, about three in any transverse section, occur each close to a bundle of the ring; they are amphivasal in structure, with xylem mostly enclosing the phloem.

The bundles of the ring have clearly been set back into line from an original scattered arrangement, being of very varying sizes and distinctly individualized.

INULOIDEAE.

Inula Helenium, L.

Stem.

In two individuals medullary strands were observed near the base and in the upper part of the stem respectively, and in one case in the base of a branch and in the peduncle. They occur near the vascular ring, and some of them are united with the sclerenchyma surrounding the xylem. They die out above. Each consists of a central strand of soft bast surrounded by lignified tissue which is probably sclerenchyma, but may be fibrous xylem. These medullary strands are distinctly rudimentary and evidently on the verge of extinction (Fig. 26).

The bundles of the ring were clearly once scattered in the ground-tissue.

Leaf.

The original vascular system of the stem of this plant is revealed, as in so many cases, by a glance at the present vascular system of the petiole. In this organ the scattered disposition of the widely-separated (tangentially and radially) bundles is obvious. They are distinctly two-ranked and of

three or four sizes, the largest being situated farthest to the outside, while the smallest, save for a few inverted ones on the immediate ventral side of one or two of the largest bundles, occur nearest the centre of the organ. Some of these smallest bundles are quite rudimentary (Fig. 27).

Further, an index to the primitive stem-structure, as regards the distribution of the main tissues, is here found in the fact that there is absolutely no distinction between cortex and pith: the two tissues are one and indivisible; the structure is astelic, a general endodermis being

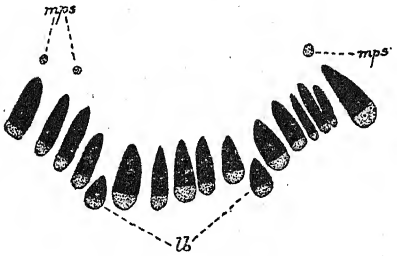


FIG. 26. *Inula Helenium.* Segment of vascular ring of stem, showing medullary phloem-strands (*mps*). Leaf-bundles at *lb*. × 9.

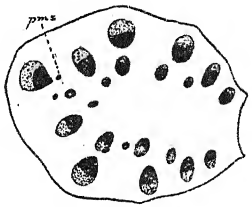


FIG. 27. *Inula Helenium.* Petiole, showing well-marked scattered disposition of the bundles, and the small inverted bundles (*pms*) on the ventral side of the median bundle of the arc. × 3.

absent, and a particular one around each bundle being present. The whole structure supports the view, with which the present writer agrees, that the pith is cortical in its origin.

SUMMARY.

The following are the main facts concerning the vascular structure of the stem and leaf in Compositae:

1. The vascular cylinder of the stem is loosely constituted, the component bundles being markedly individualized.

2. Their alinement in the vascular ring is more or less irregular, being situated at varying radial distances from the centre of the stem.

3. The stems of the vast majority of Compositae are *herbaceous*.

4. Where vascular strands occur in the medullary region of stem or leaf they, as a rule, exhibit great variety of development; some being throughout well-developed vascular bundles, others consisting of phloem only; some possessing both xylem and phloem during part of their longitudinal course, while in another part of their course the same strands possess phloem only, or have lost the lignified portion of their xylem. Many bundles dwindle in size when traced upwards or downwards, lose their xylem, then their functional phloem, and finally die out *in situ*,—in other words, end blindly in the parenchymatous tissue.

5. Within the same genus, some species possess medullary strands in the stem, while other species are completely devoid of them, e. g. *Rudbeckia*, *Lactuca*.

6. Within the same species, some individuals possess medullary strands in the stem, while other individuals are devoid of them, e. g. *Crepis biennis*, *C. virens*, *Picridium tingitanum*.[1]

7. Where the medullary system of bundles is completely or almost completely absent in the stem, it is frequently conspicuously present in the leaf, e. g. *Arctium Lappa*, *Crepis taraxacifolia*, *Senecio clivorum*.

8. There are plenty of genera in the order which do not exhibit, either in stem or leaf, the remotest trace of medullary strands; but in almost all the features referred to under (1) and (2) are present, as they are also in the leaves of *Rudbeckia*, *Tragopogon*, and *Scolymus*. The vascular system of such genera is, on the writer's view, the most advanced and has evolved out of that in which a medullary system was present.

The course of the medullary system of strands through the main stem and the peduncle might have been described in the reverse direction, viz. from above downwards. If that had been done it would have been clearly seen that, as Kruch already pointed out, some of the medullary strands arise from the *flowers*, some from the vascular ring of the lateral *branches* of the stem, others from the vascular ring of the main stem. Great numbers arise *de novo* in the pith-tissue and may either pass into the vascular ring at a lower level or may form part of a system which ends blindly, i. e. dies out *in situ* below. Some individual strands are seen to leave the vascular ring and almost immediately rejoin it at a very slightly lower level. It would also be noticed that, on approaching the extreme base of the stem, a great reduction in the number of the medullary strands occurs either by means of extinction *in situ*, as mentioned above, or by anastomosis and copious fusion until a very few large amphivasal bundles, with well-developed xylem, occur at that level. Each of these bundles is the exact morphological equivalent of the strand of internal phloem situated immediately on the inner side of each bundle of the ring at a higher level in the stem. These bundles at a lower level either pass into the vascular ring, or, after losing their xylem, die out *in situ*.

The medullary bundles are certainly not leaf-traces. The origin of some of them from flowers and branches has been stated above. Others derive from the vascular ring of the stem, which has been built up by leaf-traces; such medullary bundles may be, therefore, regarded as leaf-traces of very indirect origin. The writer could find no evidence for the view set forth, e. g. by Weiss, that a particular medullary strand may be a leaf-trace which has entered the vascular ring from a leaf at the node above, preserving its individuality through the intervening internode till it enters the pith at the node below.

The medullary strands of the stem are not continuations of those of the

[1] In *P. tingitanum* Kruch reports having found medullary strands, while the present writer could find none in the specimens he examined.

leaf, which has its own independent system. On the other hand, they are sometimes, as in the cases of *Scorzonera* and *Helminthia*, direct continuations of those of the branches.

Discussion and Conclusions.

The position assumed in this paper is that the Compositae are primitively and aboriginally herbaceous plants in habit and consistence, a view which is borne out by the fact that shrubby and arborescent forms are rare in the order. It is in this way that the writer accounts for the vascular structure of the stem and leaf throughout the order. The very irregular alinement of the bundles composing the vascular ring of the stem,[1] the presence in so many forms of medullary bundles or a scattered disposition of the ring-bundles both in stem and leaf, are brought forward as evidence in favour of the above view.

It seems to the writer a matter of considerable importance to try and determine what was the primitive type of vascular structure of the vegetative organs of the Compositae, and to endeavour to settle the question as to whether the medullary or scattered system of bundles, occurring either in stem or leaf, is a comparatively recent, adaptive structure or whether, on the contrary, it is an ancient, vestigial one. For the structure, viewed as a whole, must have one or the other origin.

What are the arguments in favour of what may be termed the 'adaptive theory'?

It has been suggested to the writer that the presence of medullary or scattered bundles in the stem may be the result of the acquirement of a congested type of inflorescence in this order known as the 'capitulum'. This particular type of vascular system would result, in the axis of the inflorescence, from the excessive shortening of the internodes in that region, combined, possibly, with a relative increase in the diameter of the axis. (As a matter of fact, it regularly occurs in the axis of the congested inflorescences of Compositae, Umbelliferae, and Dipsaceae.) The scattered system there established might then extend, in more or less accentuated form, throughout the peduncle and the vegetative stem. If, however, inhibiting factors prevailed, such as the reduction in diameter of these organs, the necessity for resisting bending-strains in an elongated axis, or the formation of a hollow pith (central lacuna), medullary bundles would either not be formed at all, or in very reduced numbers, or rarely. Further, if the scattered system occurred in the vegetative stem from the cause just mentioned this might induce, by correlation, a similar structure in the petiole of the leaf.

The scattered system of bundles would have been first laid down when the original loose spicate or racemose inflorescence first began to acquire the

[1] The reader must be again reminded that the structure is described from the transverse section.

capitulum character, and inflorescence-axis, peduncle, and vegetative stem would become dominated by this type of vascular structure. The presence of rudimentary, imperfect strands amongst the others or alone is explained on the assumption that certain factors arose, some of which are mentioned above, which induced a reduction amongst the constituents of the scattered system.

It has also been suggested to the writer that while in Compositae the scattered system of the stem may have arisen by the above method, in other Natural Orders, where a quite similar scattered system obtains, a geophytic habit may have given rise to it in the lower region of the stem, and it would subsequently extend upward into the higher regions of the axis. In the same way the scattered disposition of the bundles in the petiole may have had an origin as stated above, while in other cases it may be due to the fact that there has been no mechanical necessity in that organ for the bundles to be arranged in a ring (owing, e. g., to the relative absence of bending-strains). Moreover, the scattered system may have arisen independently in both stem and petiole; it may have already occurred in the petiole (if a fairly large organ) before the inflorescence became congested with its consequent appearance in the stem, and the scattered system in both organs would then have become mutually adjusted to produce the interdependent and continuous structure in the two organs such as we see, e. g., in the case of *Cynara*. This view involves the quasi-independent origin and development of stem and leaf.

The ideas embodied in the above statements have, doubtless, on the face of them, a certain plausibility from the point of view of those who hold that stem and leaf have an independent and coeval origin (e. g. from an aboriginal thalloid ancestor). It is a question, between the hypothesis set forth above and that of the present writer, as to which is the most reasonable and likely to be true after the widest possible survey of all the available facts. For definite proof, either one way or the other, is not forthcoming. There can be no doubt that the inquiry into the real nature and origin of the vascular system of the Compositae is a very important one in view of the attention which this order attracts amongst botanists, the number and variety of forms it contains, and its wide geographical distribution.

As already stated, the present writer holds the Compositae to be, as regards its vascular structure, a type of a very large number of Dicotyledonous orders, and the conclusions drawn in this paper with regard to the nature and origin of its vascular system will apply, once and for all, not only to this order but to all others in which scattered bundles, medullary strands, or intraxylary phloem occur. For the writer regards these phenomena as essentially the same in every one of these orders, varying only according to the idiosyncrasy of the particular order in which they occur. He has investigated too many instances to harbour any longer a doubt on

this matter. Hence the reason why it is deemed necessary to give as full and complete a discussion as possible on the whole subject while treating of the Compositae.

Now, in view of the belief just stated, and of various facts to be brought forward below, the writer regards the hypothesis of a dual origin for the scattered system[1] in the stems of Angiosperms as an extremely unlikely one. It seems much more probable that the vascular system of Angiosperms has been founded and built up on a common ground-plan, and that, therefore, the scattered system has the same origin and meaning in all.[2] If not, then an adequate explanation should be forthcoming for the presence of medullary bundles in every Natural Order in which they occur. If their occurrence in Compositae is due to the congested type of inflorescence, then one might expect a similar explanation of their presence in those Umbelliferae, e.g. *Eryngium*, which also possess a congested type of inflorescence. As a matter of fact the scattered system obtains in the axis of the inflorescence of this genus. But the vegetative habit and external conformation of some species of this genus, e.g. *E. Serra*, is indistinguishable from that of a Monocotyledon, and the scattered system of bundles in their stem and leaf almost entirely resembles that in the stem and leaf of Monocotyledons. Hence the conclusion may fairly be drawn that the vascular structure in the stem and leaf of these plants has the same meaning and origin as it has in Monocotyledons, viz. a result of the geophytic habit, and is not an extension downward of that obtaining in the axis of the inflorescence, which, on the adaptive theory, is for the purpose of supplying the crowded florets. In this connexion it may be pointed out that in the axis of the inflorescence of *E. amethystinum* there is a system of medullary bundles which have nothing whatever to do with the supply of the florets, for they die out both above and below without forming connexions with the peripheral bundles, and therefore are clearly vestigial structures, having been retained in this region where bending-strains are absent, while, on the writer's view, they have been purposively eliminated from the peduncle and vegetative axis of this species.

In other, less Monocotyledon-like genera of Umbelliferae, e.g. *Ferula*, whose inflorescence is not congested like it is in *Eryngium*, the scattered system of the stem is equally well marked. It may be admitted that the complete absence of the scattered system in the stems of Dipsaceae[3] and Proteaceae, both of which have congested inflorescences, may be due to the development of a hollow pith in the stem of some members of the former and to the woody, xerophytic habit of the stem in the latter. But

[1] In this part of the paper 'scattered system' and 'medullary bundles' stand for the same phenomenon.

[2] An exception must, perhaps, be made for the important group of the Amentiferae, which may have a distinct origin from other Dicotyledonous orders.

[3] It is present in the axis of the inflorescence of this order.

this seems an inadequate excuse for their absence in Dipsaceae, which ought, one would think, to exhibit this feature if it is primarily due to the congested inflorescence, for in a stem of *Morina elegans* investigated by the writer there is a very wide solid pith, yet medullary strands are absent.

Again, medullary strands are a very marked feature of the Melastomaceae, occurring in numerous genera, yet there is no congested inflorescence in this order, nor numerous floral parts, nor is there any other special character in the order which may account for their presence, unless it be the ring formed by the continuous bundles in the stem, ensuring adequate resistance to bending-strains, and therefore precluding the necessity of a hollow pith with the consequent elimination of medullary strands; yet this cannot be the explanation, for *Lasianthus strigosus*, with a similar vascular ring, possesses no medullary strands. If the Melastomaceae have descended from arboreal or shrubby forms (which is the view generally held) the medullary strands would be, probably, a recently-acquired feature and adapted for some special function. But it is very difficult to see, on this view, for what function they have been acquired, why they occur in some forms and not in others, and why they are well-developed in some places and rudimentary in others.

Again, to take at random two instances which are typical of others, why should the almost precisely similar intraxylary phloem-strands in the stems of *Tragopogon* (Compositae) and *Nicotiana* (Solanaceae) be supposed to have two quite different origins? For in Solanaceae a congested type of inflorescence does not occur; hence some other cause for the presence of intraxylary phloem in this order must be found than that which is held, on the above view, to account for its presence in Compositae. Yet, in the present writer's opinion, it is more than probable that a structure which is identical in both orders must have had the same origin in each.

Nor, indeed, has the occurrence of phloem in the pith, such, e.g., as obtains in the Gamopetalae, ever yet been explained on purely adaptive grounds. Why phloem should occur in this (for it) wholly abnormal situation is a problem which requires solution, but which no anatomist has hitherto attempted to solve!

The *sporadic* occurrence of medullary bundles both in Compositae and other Natural Orders can with difficulty be accounted for on the theory which is opposed to that of the present writer. The case of *Rudbeckia* may be taken as an illustrative one. It might be assumed that the medullary bundles of *R. maxima* are retained owing to the greater diameter of the stem of that species as compared with that of most of the others, and that their presence in *R.* (*Echinacea*) *purpurea* is owing to the absence of a lacuna in the pith. But these differences in the stems of the various species appear to the writer to be insufficiently great or important, and therefore inadequate to account for the sporadic occurrence of the medullary bundles on the view

that these are to be regarded as a comparatively new and adaptive feature. It is, on this view, still more difficult to account for their presence in the stems of some species of *Lactuca*[1] and their complete absence in other species, and the writer is unable to discover any adaptive features which should explain this remarkable fact.

Again, the writer cannot agree that the scattered disposition of the bundles in the *petiole* (where it occurs) can have two distinct modes of origin; here also, there must be a single underlying cause for the phenomenon not only in the Compositae, but in all other Dicotyledons which exhibit it. A survey of *all* the facts strongly points to this conclusion.

The hypothesis, as stated above, that the scattered system may have had a quite independent origin in stem and leaf is, on the view that these organs are quasi-independent the one of the other, a quite possible one. But, having regard to all the facts in all Natural Orders concerned, it is a very inadequate hypothesis, especially in view of the intimate connexion and relationship between stem and leaf. A single fact which is detrimental to this view may be cited, viz. the continuation of typical leaf-structure, in so many cases, into the cortex of the stem.

The theory held by the writer is that the leaf, in its anatomical organization, is identical with that of the stem; that it has an epidermis, cortex, vascular system, and pith which are morphologically identical, and aboriginally one and continuous, with the similar tissues of the stem. The morphological and anatomical history of the two organs are inseparably bound up together. Further, the leaf is a more conservative organ than the stem. To cite a single physiological fact: the leaf has not been obliged, to the same extent as is the case with the stem, to adapt its structure for the resistance of bending-strains. This being so, and if the medullary system of strands of the stem represents, as seems obvious for the reasons set forth above, an ancestral character, then we should expect this system to be more frequently present in the leaf than in the stem or, if it occurs in the stem, that it would often occur in a better developed condition in the leaf. This is, in fact, exactly what we find.

In the stem of *Senecio* medullary bundles are practically extinct—only extremely rudimentary traces of them were observed in *S. Petasites*. Yet in the petiole, for example, of *S. clivorum*, medullary bundles represent an extremely well-developed system. A similar state of things exists in the case of *Inula Helenium*.

In the genus *Crepis* medullary bundles are either absent or very rare in the stem; whereas in the petioles of *all* species examined by the writer they are present in considerable numbers, although in a very rudimentary

[1] They probably have some use, or they would not have been preserved; but it is much more easy to account for their distribution in this genus on the vestigial theory than on the theory that these strands are recently-acquired adaptive structures.

form, the adaxial portion of the vascular ring being frequently also in a correspondingly rudimentary state (Fig. 11).

The genus *Lactuca*, as regards the occurrence of medullary strands in stem and leaf, exhibits interesting variations. *L. Plumieri*, e.g., has almost lost them in the stem, whereas they are abundant in the leaf, although in a rudimentary form.

In *L. macrophylla* they are completely absent in the stem, and only the slightest remnants of them can be found in the leaf, a fact which is obviously due to the presence of a very large lacuna in the pith. Where the lacuna is quite small or absent, medullary strands are usually present, as in *L. Plumieri* and *L. hastata*; in the last-mentioned species medullary strands are entirely absent from the stem. In *L. Scariola* medullary strands are exceedingly well developed in the stem; in the leaf they are quite absent.

This last fact shows that, although the leaf is a more conservative organ than the stem, there are cases in which its vascular system has become more reduced than that of the stem. The medullary strands in the stem of *L. virosa*, *L. saligna*, *L. Scariola*, and *L. sativa* have been *retained* in order to subserve some useful function; they are not merely vestigial but, in all probability, also adaptive structures.[1]

In the leaf no necessity for their retention exists, and at the same time this organ, as compared with that of *L. Plumieri* and *L. alpina*, has become reduced in size. The same phenomenon recurs in the case of *Rudbeckia*. In some species medullary strands occur in the stem, in other species they are absent. But in the leaf of all species except *R.* (*Echinacea*) *purpurea* they are completely absent, the entire vascular system having been reduced to a comparatively short arc of bundles, a fact which is probably due to the petiole having been relatively reduced in size.[2]

A striking example again is afforded by *Tragopogon*. The stem of all species has very numerous medullary strands. The leaf has become much reduced and almost grass-like in its organization, in accordance with which fact the medullary strands, in the case of *T. pratensis*, have become quite extinct in the leaf, while in the case of the other species investigated they are small and insignificant as compared with those of the stem, dying out in the leaf-base.

The theory, therefore, is that in Compositae the vascular structure of the leaf, wherever it is large and well developed, constitutes a reliable index to the fact of the former universal presence of medullary strands in the stem. But it must be taken in conjunction with the fact of the prevalently imperfect, rudimentary character of the medullary system of the stem (wherever this occurs), and with the phyton-theory as a basis (viz. that

[1] This must be true in all cases where the medullary strands consist of functional tissues.

[2] The lamina may be of quite large size.

stem and leaf are really one and indivisible, the former being built up of leaf-bases). The theory undoubtedly receives confirmation from a study of the vascular system in other Natural Orders.

In Polygonaceae the genus *Rumex* contains some species which possess in the *stem* a medullary system of strands, always in a more or less imperfect, rudimentary form; while in the stem of other species it is completely, or almost completely, absent. But in the *petiole* of the leaf of *all* species, with the possible exception of the small, reduced *R. Acetosella*, medullary bundles occur, not in an imperfect, rudimentary, but in a very well-developed condition. This uniformity in the vascular structure of the leaf as compared with the variableness and inconstancy of that of the stem is strongly in favour of the view that the vascular structure of the leaf represents, of the two systems, the more ancient type, that, viz., in which a well-pronounced, scattered system of bundles occurred in both stem and leaf.

In Umbelliferae there are a large number of cases in which, where the stem exhibits no trace of medullary strands, they are well developed in the petiole. The markedly imperfect, rudimentary nature of the medullary system of the stem, in the majority of cases where it occurs, clearly shows this feature to be an ancestral, vestigial one, which, in many species, has been preserved in its pristine condition in the more conservative foliar organs.

The adaptive theory, as set forth on an earlier page, cannot account for the very frequent presence of rudimentary, imperfect strands both in stem and petiole. If they represent a comparatively recent acquisition resulting from the congested type of inflorescence, then the very widely-diffused presence in Compositae of the scattered system (or medullary strands) in an imperfect condition of development can with difficulty be accounted for and is inconsistent with its having been recently acquired; there should be, one would think, some very definite explanation thereof forthcoming. The writer, however, knows of no adequate explanation of the phenomenon from the point of view of the theory opposed to his own.

On the writer's view, there must be one single theory to cover all the facts of the case, which is essentially the same for all the Natural Orders concerned; a single theory to account for the medullary system in both stem and leaf in whatever condition, perfect or imperfect, it may occur.

The writer is acquainted with but one theory which will accomplish this, viz. that the Compositae, as also the great majority of Dicotyledonous Natural Orders, are derived from a 'Monocotyledonous' or geophytic stock in which the grandifoliate character prevailed, the leaf dominating the stem, and the latter being primarily built up of leaf-bases. The two organs, stem and leaf, would be thus in reality one and indivisible, and their vascular structure identical, such as we see in the typical Monocotyledon of the present day.

Although all types of inflorescence and all kinds of vegetative habit are found in the Monocotyledons, yet no one has ventured to suggest that the scattered disposition of the bundles in stem and leaf is due to more than a single cause, that cause being recognized as lying in the geophytic habit (modern or aboriginal) of the plants composing the class. In the same way, it is more than likely that there must be a single cause for the same type of vascular system of the closely-allied class of Dicotyledons.

Just as we regard the two great classes as having had a common origin in the past, in the same way we must regard the vascular system of each as being derived from a common ancestral type of vascular system.

The question therefore arises, What has this ancestral type of vascular system been? Has the structure of Compositae (a type of most other Dicotyledonous orders) been derived from that which consisted of a scattered disposition of the bundles in stem and leaf, or from that which consisted of a ring of bundles (as seen in transverse section) in the stem and either a ring, or arc, of bundles in the leaf? Another form of the question is: Were the ancestors geophytes, or were they non-geophytic herbaceous or arboreal forms?

A comprehensive survey of all the facts clearly suggests that the ancestors were either geophytes or semi-geophytes. The Compositae and the other Dicotyledons are now emerging from this ancestral condition; a small minority of Compositae have, indeed, actually become arboreal in habit; but the herbaceous habit is certainly primitive in this order, as most systematists who are conversant with it will probably agree.

Jeffrey gives an account, in his chapter entitled 'The Herbaceous Dicotyledons', of the structure of the vascular ring of the stem of Compositae at the point where the leaf-trace is detached. He compares the structure of the more woody and the more herbaceous types respectively. He concludes, in effect, that the facts constitute clear evidence that the herbaceous type of structure has been derived from the woody type. The present writer desires, however, to state most emphatically his complete inability to discover any evidence whatsoever in the facts set forth by Jeffrey with regard to the structure of Compositae, or any other order, which would tend to indicate that the herbaceous type of structure has been derived from the woody type. The entire chapter is devoid of scientific argument, consisting of scarcely more than a descriptive statement of the structural facts.

If the view be held that the Compositae are primitively herbaceous, then the sporadic occurrence of medullary bundles, their presence in some individuals, species, or genera, and not in others, their predominantly rudimentary, imperfect development and structure, as also their late ontogenetic appearance as compared with that of the main vascular system, all become

easily explicable; for these are the very features which always characterize vestigial, ancestral structures.

The writer does not feel called upon, for the purposes of the present thesis, to determine the precise factors which govern the sporadic distribution of the medullary strands: why, for example, they occur in the stem of *Lactuca saligna* and not in that of *L. macrantha*. The fact by itself is all-important and self-sufficient. To cite comparable cases: the presence of staminodes in the flower of some Primulaceae (e.g. *Samolus*) and not in others, and the presence of inflorescence bracts in some genera of Cruciferae and their complete absence in others. It is enough that these facts indicate that an outer whorl of stamens and inflorescence bracts were once constant and normal features respectively in the ancestors of these plants.

Having now weighed all the facts and hypotheses bearing on the question, the writer arrives at the final conclusion that the intraxylary phloem occurring in the stem of Compositae (a type of many other orders) is the vestige of a formerly well-developed system of medullary bundles which, along with the bundles of the vascular ring, constituted a scattered system of bundles, which was the normal feature of the stem in every member of the order, or its ancestry, at a time when the geophytic or semi-geophytic habit prevailed in these plants. The more conservative foliar organs very often retain the ancestral structure, more or less well preserved, when it has become extinct, or almost so, in the stem.

The writer is indebted to the authorities of the Royal Gardens, Kew, for the greater part of the material used in this investigation. He also desires to thank Mr. L. A. Boodle, F.L.S., for some critical suggestions, and for taking part in numerous discussions, affecting certain aspects of the theory embodied in the paper.

Bibliography.

Barratt, Miss K.: A Note on an Abnormality in the Stem of *Helianthus annuus*. Ann. of Bot., vol. xxx, July, 1916.

Col: Recherches sur la disposition des faisceaux dans la tige et les feuilles de quelques Dicotyledones. Ann. Sci. Nat., Bot., Ser. 8, vol. xx, 1904.

Jeffrey, E. C.: The Anatomy of Woody Plants, 1917, pp. 386–408.

Kruch: Fasci midollari delle Cichoriaceae. Ann. R. Ist. bot. di Roma, vol. iv, fasc. 1, 1890.

Peter: Der anatomische Bau des Stengels in der Gattung *Scorzonera*. Nachr. v. d. Königl. Gesellsch. d. Wiss. zu Göttingen, Heft 1, p. 9, 1898.

Petersen: Ueber das Auftreten bicollateraler Gefässbündel in verschiedenen Pflanzenfamilien und über den Werth derselben für die Systematik. Englers Bot. Jahrb. f. syst. Pflanzengesch. und Pflanzengeogr., vol. iii, p. 359, 1882.

Weiss, J. E.: Das markständige Gefässbündelsystem einiger Dikotyledonen in seiner Beziehung zu den Blattspuren. Bot. Centralbl., vol. iii, 1883, p. 283.

Whitaker, Miss E.: Anatomy of certain Goldenrods. Bot. Gaz., vol. lxv, March, 1918, p. 259.

Studies on Intrafascicular Cambium in Monocotyledons (III and IV).[1]

BY

AGNES ARBER, D.Sc., F.L.S.,

Fellow of Newnham College, Cambridge.

With seven Figures in the Text.

CONTENTS.

	PAGE
III. ON THE DEVELOPMENT OF THE FOLIAR BUNDLES IN *Veratrum album* L.	459
IV. NEW RECORDS OF THE OCCURRENCE OF INTRAFASCICULAR CAMBIUM	462
METHODS	464
SUMMARY	464
ACKNOWLEDGEMENTS	465

III. ON THE DEVELOPMENT OF THE FOLIAR BUNDLES IN *VERATRUM ALBUM* L.

THE part of the vegetative shoot of *Veratrum album* L. visible above ground during the spring and summer appears at first glance to consist of an erect axis bearing spirally arranged sessile leaves. But on closer examination the 'axis' proves to be merely the extremely long cylindrical leaf-sheaths, which successively enclose one another, the true stem being subterranean. The life and growth of the leaves are markedly protracted. If the plant be examined towards the end of August, when—at least in plants cultivated in this country—the leaves of the current year are fully mature and already beginning to fade, next year's leaves will be found beneath the level of the ground, enclosed in a flask-like dilatation of this year's leaf-sheaths. The outermost of next year's leaves take the form of closed conical scales, and surround the normal foliage leaves with their short sheaths and delicately plicate blades.

In the mature leaves, gathered towards the end of July, each of the main bundles of the lamina, as seen in transverse section, is surrounded by

[1] These studies form a continuation of:—

I. Arber, A. (1917): On the Occurrence of Intrafascicular Cambium in Monocotyledons. Ann. Bot., xxxi, p. 41.

II. Arber, A. (1918): Further Notes on Intrafascicular Cambium in Monocotyledons. Ann. Bot., xxxii, p. 87.

a fibrous sheath (Fig. 3, *f.*), interrupted in the region between the xylem and phloem. The phloem is remarkable for the arrangement of its elements in extremely long radial files—an evidence of an unusual degree of cambial activity. The xylem consists of three highly distinct types of element—protoxylem (*px.*), large lumened metaxylem (*mx.*) and, on the side towards the phloem, a zone of elements of much smaller calibre with scalariform sculpturing (xy_2.). This occurrence of relatively narrow, lignified elements, adjacent to the larger metaxylem vessels on the phloem side, is not infrequently seen in transverse sections of the leaves of Monocotyledons, though it is seldom so strikingly displayed as in *Veratrum album*. I have observed it, for instance, in varying degrees, in examining sections of the following species:

LILIACEAE.

Albuca nelsoni N. E. Br.
Allium fistulosum L. (Fig. 4).
A. porrum L.
A. ursinum L.
Anthericum sp.
Arthropodium paniculatum R. Br.
Colchicum byzantinum Ker-Gawl.
Hemerocallis fulva L.
Hyacinthus (garden var.).
Kniphofia caulescens Baker.
Phormium tenax Forst.
Scilla hispanica Mill.
Smilax laurifolia L.
Veratrum album L. (Fig. 3).

HAEMODORACEAE.

Anigozanthos sp. (Fig. 5).

AMARYLLIDACEAE.

Crinum sp.
Cyrtanthus sanguinea Walp.
Narcissus pseudo-narcissus L. (garden var.).

IRIDACEAE.

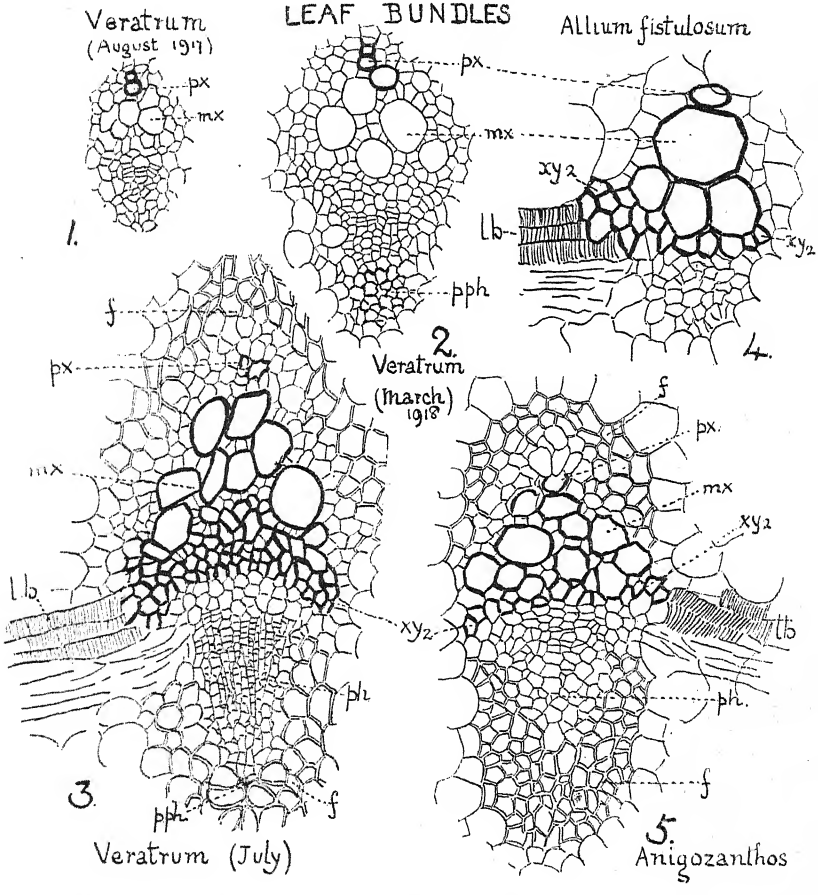
Crocus (garden var.).
Iris orchioides Carr.
I. sisyrinchium L.
I. sp.
Moraea polystachya Ker-Gawl.

ZINGIBERACEAE.

Hedychium sp.

In most of the cases enumerated in the above list it is difficult, owing to

the irregular disposition of the small xylem elements in question, to say with certainty how far they are the result of cambial activity and how far sliding growth may be a factor in their origin. Some obscurity exists on this point in the case of *Veratrum*. But in *Anigozanthos* sp. (Fig. 5), *Kniphofia caulescens*, *Phormium tenax*, and *Iris* sp., I have seen clear indications that these elements are truly secondary and are derived from the cambium.



FIGS. 1–5. Transverse sections of leaf-bundles (× 190 *circa*). *f.* = fibres; *px.* = protoxylem; *mx.* = *metaxylem*; xy_2. = secondary xylem; *p.ph.* = protophloem; *ph.* = phloem; *l.b.* = lateral bundle. Figs. 1–3. Development of bundle in leaf-blade of *Veratrum album* L.; Fig 1, from young leaf for next year still enclosed in terminal bud (August 22); Fig. 2, from leaf beginning to develop in succeeding spring (March 22); Fig. 3, from mature leaf (July 28). Fig. 4. *Allium fistulosum* L. Fig. 5. *Anigozanthos* sp. Figs. 3–5 show the mode of attachment of the xylem of a branch vein (*l.b.*) to that of the bundle from which it arises.

Lateral veins are a relatively inconspicuous feature in the leaves of most Monocotyledons, but there seems to be some evidence that where they occur in species showing this differentiation of the xylem, their tracheal elements tend to be connected exclusively with the *secondary* wood of the

longitudinal veins. This is conspicuously the case in *Veratrum* (Fig. 3, *l.b.*), and I have noticed the same thing in some of the other Liliaceae—*Allium* (Fig. 4), *Kniphofia*, *Hemerocallis*, and *Anthericum*—and in *Iris orchioides*. But I have not investigated the point fully enough to generalize upon it. In one case in *Anigozanthos* I observed a lateral vein whose xylem, though attached in part to the secondary elements, was also connected with the metaxylem ; but in the bundle drawn (Fig. 5) the connexion was apparently with the secondary xylem alone.

Apart from the differentiation of the xylem, the leaf-bundles of *Veratrum* are of special interest as furnishing a conspicuous case of what we may call *deferred cambial activity*. The cambium formed in one season survives the winter, and is operative in the succeeding year. Fig. 1 shows a bundle belonging to a leaf taken in August from next year's bud ; here cambium is already visible. Fig. 2 represents the stage reached by a similar bundle in March of the following year, when the cambium is actively dividing, while Fig. 3 shows the bundle in July when it is fully mature and all the work done by the cambium can be recognized. A Monocotyledonous bundle in which cambium is active in two succeeding seasons has hitherto been described in only one case—that of the tubers of *Gloriosa superba* L.[1] But no doubt other examples will come to light when organs which persist through two or more years are examined from this point of view.

IV. New Records of the Occurrence of Intrafascicular Cambium.

Since the publication of my former list of Monocotyledonous families[2] in which the occurrence of cambium has been recorded, I have observed it in the following additional cases

Juncaceae.

Juncus glaucus Sibth. In the leaf-bundles there is distinct cambial activity, which seems—as is generally the case in the related Liliaceae—to be chiefly directed to phloem production.

Haemodoraceae.

Anigozanthos sp. Cambium occurs in the leaf-bundles and gives rise to both xylem and phloem elements (Fig. 5).

Amaryllidaceae.

Narcissus pseudo-narcissus L. (garden var.). In the mature leaf, traces can be detected of a cambium which gives rise to phloem—possibly also to xylem elements.

[1] Queva, C. : Contributions à l'anatomie des Monocotylédonées. I. Les Uvulariées tubéreuses. Trav. et Mém. de l'Université de Lille, T. 7, Mém. xxii, 162 pp., 11 plates, 1899.

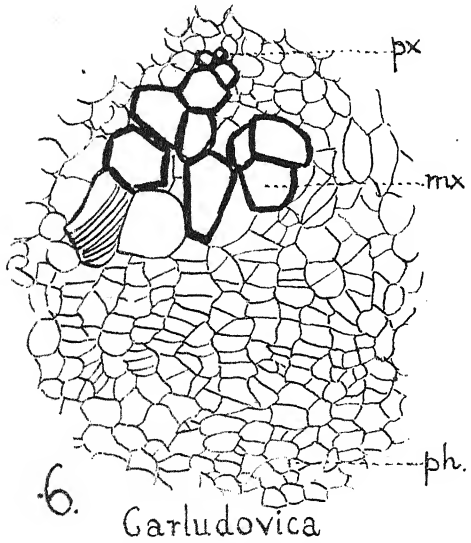
[2] Arber, A. (1918), l.c.

IRIDACEAE.

Crocus sp., *Iris* sp., *Moraea polystachya* Ker-Gawl., and *Sisyrinchium* sp. Since in my previous paper[1] cambium in this family was only recorded in one case (*Tritonia*), it may be worth while to note here its occurrence in the leaves of members of these four additional genera.

CYCLANTHACEAE.

Carludovica rotundifolia H. Wendl. The vascular bundles of the axis of this plant, in the region near the growing apex, show cambial activity which, though somewhat irregular, is quite well marked (Fig. 6). This case is of interest because there has hitherto been no record of cambium from this Cohort (Synanthae).



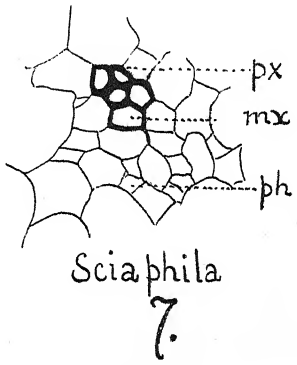


FIG. 6. *Carludovica rotundifolia* H. Wendl. Transverse section of bundle from near growing point of axis (× 258 *circa*). FIG. 7. *Sciaphila purpurea* Benth. Transverse section of a bundle from an axis less than 1 mm. in diameter (× 424 *circa*). *px.* = protoxylem; *mx.* = metaxylem; *ph.* = phloem.

TRIURIDACEAE.

I have examined two species of *Sciaphila*, as representatives of the small Cohort of the Triuridales. This saprophytic genus has leaves reduced to little scales, and the development of the vascular tissue is correspondingly slight. Fig. 7 represents a bundle from the transverse section of an axis of *Sciaphila purpurea* Benth., which was less than 1 mm. in diameter. The arrangement of the elements may perhaps be interpreted as indicating a vestigial trace of cambial activity.

[1] Arber, A. (1918).

Methods.

In cases where it has been necessary to use dried herbarium material for sectioning, I have employed the method of preparation devised by Dr. R. C. McLean.[1] The basis of this method is prolonged treatment with dilute solutions of caustic potash. For purposes of economy I substituted impure waste spirit (less than 90 per cent.) for the absolute alcohol recommended by McLean for the first stages of his process, and I also dispensed with an air-pump, but in spite of these modifications the method gave most successful results, even with such delicate structures as the inflorescence axes of the Triuridaceae. The treatment of the material took place in a room which was kept at such high temperatures that the fluids nearly always felt tepid to the touch, and I think that this continuous warmth probably assisted the recovery of the tissues considerably.

Summary.

III.

Attention is drawn to the development of the leaf-bundles in *Veratrum album* L., because this is the only case among Monocotyledons (except that of the tubers of *Gloriosa superba* described by Queva) in which an intrafascicular cambium formed in one year has been observed to persist through the winter and to function in the succeeding year. These bundles also show a clear differentiation between primary metaxylem and secondary xylem consisting of a group of elements of smaller calibre. The same differentiation has been observed in varying degrees in a number of other Monocotyledons. There is some evidence that it is usual for the xylem of the lateral veins to be attached exclusively to the *secondary* xylem of the bundles from which they arise.

IV.

The existence of intrafascicular cambium is recorded for the first time in the Juncaceae, Haemodoraceae, Amaryllidaceae, and Cyclanthaceae. The Monocotyledonous families in which it is known now number nineteen. I pointed out in 1918 that cambium had then been recorded in nine out of the eleven Cohorts of Monocotyledons. In the present paper I have been able to establish its existence in a tenth Cohort, the Synanthae. In the case of the extremely reduced bundles of the eleventh Cohort—the Triuridales—the arrangement of the elements suggests a vestigial trace of cambial activity. This group can therefore scarcely be regarded as forming an exception to the general rule that intrafascicular cambium occurs in all the Cohorts of the Monocotyledons.

[1] McLean, R. C.: The Utilization of Herbarium Material. New Phyt., xv, 1916, pp. 103–7.

ACKNOWLEDGEMENTS.

I wish to express my gratitude to the Director, and to the Keeper of the Herbarium, the Royal Botanic Gardens, Kew, for material of *Carludovica* and the Triuridaceae, and also to Professor Seward, F.R.S., for the opportunity of examining members of the latter family in the Cambridge Botany School Herbarium. I am indebted to Dr. Schonland of Grahamstown for kindly sending me leaves of *Moraea polystachya* and to Mr. R. Irwin Lynch, M.A., Curator of the Cambridge Botanic Garden, for all the rest of the material examined.

BALFOUR LABORATORY,
CAMBRIDGE.
November 7, 1918.

The Cytology of the Cladophoraceae.

BY

NELLIE CARTER, D.Sc.

With Plate XXVII and two figures in the Text.

ALTHOUGH the nature of the cell-wall and branching of *Cladophora* and *Rhizoclonium* has recently been investigated by Brand (1895, 1898, 1899, 1901, 1902, 1904, 1906, 1908, 1909), and a systematic account of the genus *Rhizoclonium* has been given by Stockmeyer (1890), no exact information has been obtained concerning the cytology of the Cladophoraceae, and with the exception of the rather scanty observations made by Borzi (1883), Schmitz (1879, 1882), Gay (1891), and others, and the more recent work of Wille (1901) on *Rhizoclonium*, very little is known of the internal structure of the frequently very large coenocytes which form the thallus in species of this family.

In Kützing's original descriptions of the three genera *Chaetomorpha*, *Rhizoclonium*, and *Cladophora*, no information at all is given concerning the internal structure of *Chaetomorpha*, whilst for *Cladophora* he gives the description, 'Substantia gonimica primum cryptogonimica, effusa, demum granulosa et amylacea, saepissime in lineas laxe spirales ordinata', and for *Rhizoclonium*, 'substantia gonimica viridis subtiliter granulosa et aequaliter diffusa'.

Schmitz (1879) noticed the variability of the chloroplasts in *Cladophora glomerata* and *Cl. fracta*, and remarks that in these two species and also in *Chaetomorpha* the cell-cavity is usually traversed by an internal network of protoplasm, and that on some occasions chloroplasts are also contained in these internal protoplasmic strands. In a later work (1882) Schmitz figures the chloroplast of *Cladophora arcta* as a much perforated parietal sheath, and says that in other species of the genus the chloroplast is much more perforated and lobed, and from it arise numerous band-like outgrowths which penetrate the interior of the cell and fill it with a coarse green network. He also observed that in many members of the Siphonocladiaceae, including species of *Cladophora*, the single parietal cylinder seems to have been divided to form numerous small plates of varying size and form.

Strassburger (1880) also found that the chloroplast in *Cladophora* consisted of a more or less netlike parietal sheath or else plates of irregular

[Annals of Botany, Vol. XXXIII, No. CXXXII. October, 1919.]

outline, whilst Gay (1891) also figures coarsely reticulated chloroplasts in *Cl. fracta* and *Rhizoclonium hieroglyphicum*.

Kjellmann (1897) gives an account of the chloroplast of *Aegagropila canescens*, which he says consists of a cylindrical parietal sheath, from which arises a network penetrating into the interior of the cell and consisting of very thin, almost colourless lamellae bound up in a cell-like mass. Kjellmann gives figures to illustrate this internal network, and it seems to be rather different from the coarse network described by Schmitz (1882), neither has any structure comparable with it been observed during the present work.

Brand (1899, 1901, 1906 A) remarks on the form of the chloroplast in *Cladophora*. In the first paper he discusses the occurrence of chloroplasts in the form of spiral bands as described by Kützing (1843) for the summer condition of *Cl. fracta*, and Roth (1797–1806) for *Cl. crispata*, and since he himself had never observed such chlorophyll bands, comes to the conclusion that this appearance was not normal. In the second paper he remarks that in all the freshwater species of *Cladophora* he had examined, the characters of the chloroplast were similar to those described by Schmitz (1879, 1882), Strassburger (1880), and Kjellmann (1897), with the exception of the internal network, which could only be demonstrated in certain cases. Brand never observed a nearly uninterrupted parietal sheath such as was figured by Strassburger (1880), and is also of the opinion that the very thin drawn-out networks, and also the completely free small plates described by earlier investigators, are only found in extraordinary circumstances; the former condition being general in cells which have become abnormally lengthened by long exposure to strong light, and the latter in cells turned away from the light and about to form rhizoids. In the last-mentioned paper Brand again comments on the so-called spiral chloroplasts of *Cl. crispata*.

The genus *Rhizoclonium* has been investigated by Wille (1901), who finds that a peculiar feature of *Rh. riparium* is the presence of numerous short outgrowths proceeding from the external surface of the chloroplast towards the cell-wall. In *Rh. Kerneri* these outgrowths were much smaller, and in neither species could they be distinguished in the fixed and stained condition. Wille seems to think that these outgrowths of the chloroplast are obliterated during the process of fixation.

With regard to the number of nuclei, it has generally been believed that the segments of *Cladophora* and *Chaetomorpha* contain very numerous nuclei, whereas in *Rhizoclonium* they are very reduced in number. Thus Borzi (1883) found that in *Rh. hieroglyphicum* the shortest cells contained only one nucleus, whilst in the longer segments there were sometimes as many as four, and Gay (1891) in one instance found five. Wille (1901) never observed more than four nuclei in either *Rh. riparium* or *Rh. Kerneri*.

Brand (1898) considers the question of the number of nuclei in the Cladophoraceae and comes to the conclusion that it is not the character of the genus and the length of the cells which are important in this respect, as was formerly believed, so much as the cubical content of the cell. In a later work Brand (1909 B) points out that in certain conditions, for example in the 'statu subsimplex', certain thin varieties of *Cladophora* may only have one or few nuclei in each segment, so that the number of nuclei cannot be used as a systematic distinction between the two genera *Rhizoclonium* and *Cladophora*.

Thus the cytology of the larger members of the Cladophoraceae has not been thoroughly investigated and there has long been some doubt with regard to their exact nature. The statements of Schmitz (1879, 1882) and Kjellmann (1897) that the chloroplast is not confined to the parietal positions but also penetrates into the interior of the cell have been doubted by Brand (1901, 1902), and it was felt that the only means of obtaining accurate information was by cutting sections. Professor G. S. West therefore suggested that this work should be undertaken.

Methods. For the Cladophoraceae the best fixing solution was found to be corrosive sublimate, 3 grm., glacial acetic acid, 1 c.c., and 50 per cent. alcohol, 100 c.c. The solution was used cold, and was allowed to act for about 30 seconds. The solution used for the fixing of Desmids, which was exactly similar but contained 3 per cent. acetic acid, was found to have a too violent action on the cell-wall for it to be satisfactory in the case of *Cladophora*; cf. Brand (1901). The subsequent treatment was similar to that used in the case of Desmids.[1]

Some of the material was embedded in paraffin and sectioned, since, owing to the large size of the cells and the dense nature of the cell-contents, particularly in the autumn condition when starch is accumulated in large quantities, it is quite impossible to come to any conclusion about their structure from mere superficial observation.

Cytology.

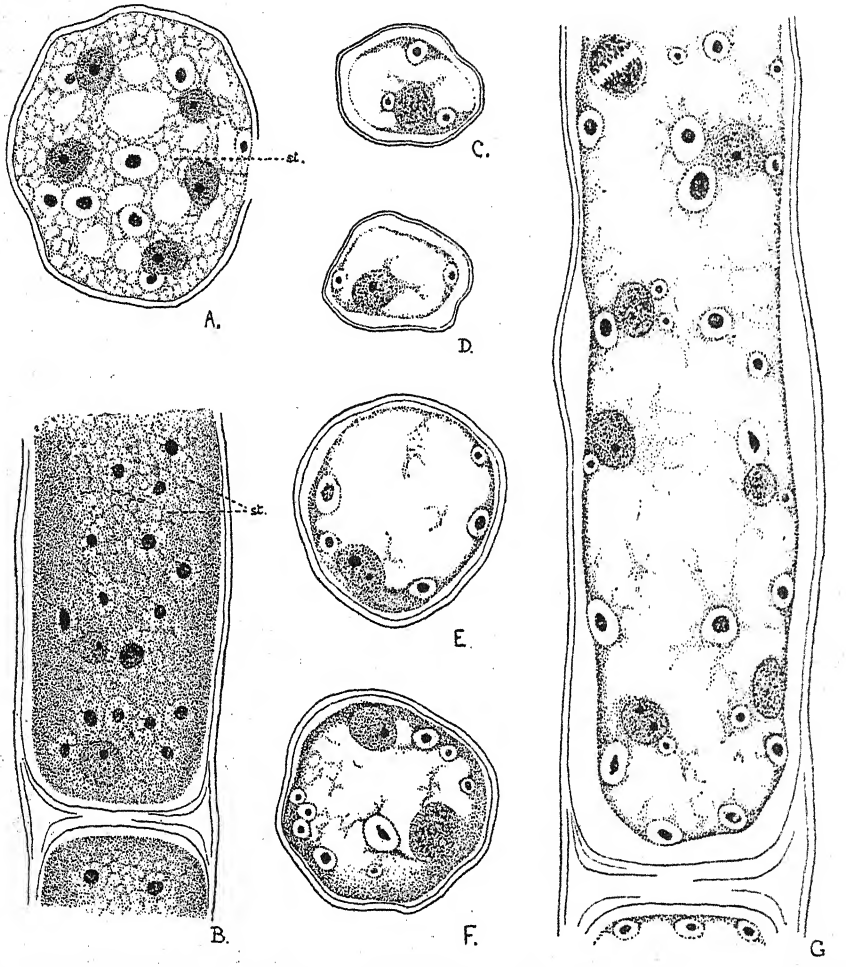
The algae examined were freshwater species of *Chaetomorpha*, *Rhizoclonium*, and *Cladophora*, since only these were available.

The cytology of these genera was found to be quite similar, although the exact condition of the segments was liable to variation according to the relative profuseness of the cell-contents.

The chloroplast invariably consists of an extensive parietal sheath mantling the whole cell-wall, including the transverse septa, and which in segments with plentiful contents forms an almost uninterrupted layer (Text-fig. 1, B).

[1] Carter, N.: Studies on the Chloroplasts of Desmids. I. Ann. Bot., xxxiii, 1919.

Where the cell-contents are not quite so profuse, however, this external layer is often more or less perforated, so that considerable patches of the cell-wall are left uncovered (Pl. XXVII, Fig. 3). In Text-fig. 1, C, a segment of this kind is seen in section, and it is quite clear that the chloroplast does not

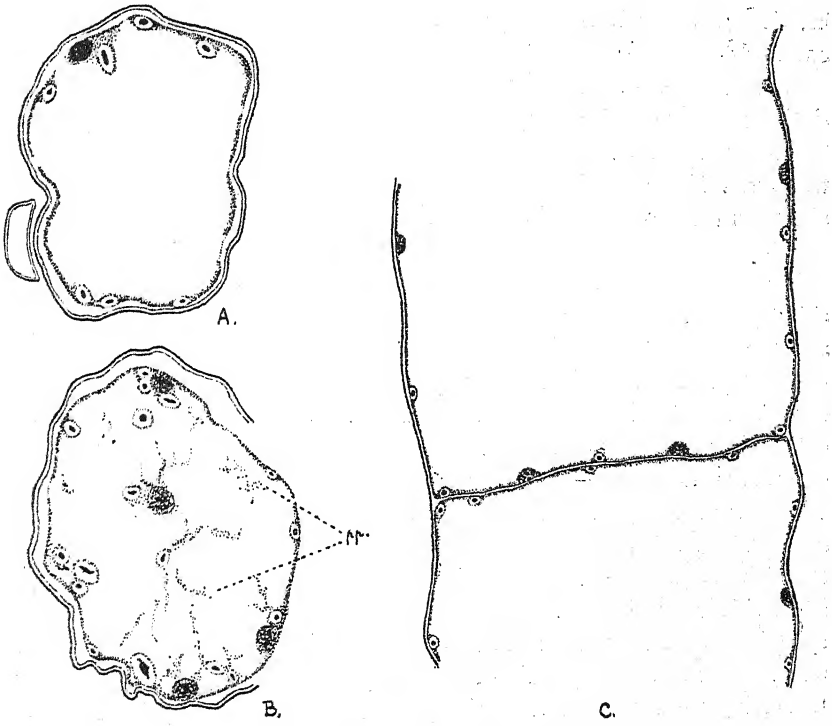


TEXT-FIG. 1. *Rhizoclonium hieroglyphicum*, (Kütz.) Stockm. A, transverse section of a filament in the autumn condition; B, external view of part of a similar filament; C and D, transverse sections of a very narrow form; E and F, transverse sections of a much larger form; G, part of a longitudinal section of a similar filament; *st*, starch-grains. All × 910.

cover the whole cell-wall. The size of the perforations depends entirely on the amount of chloroplast available in the particular segment, and therefore often varies in different parts of the same thallus. This is particularly noticeable in *Cladophora glomerata*, (L.) Kütz., in which the younger apical segments are often densely filled with cell-contents, and here the cell-wall is usually completely mantled by the chloroplast, whilst in the lower cells

of the branches the parietal layer of chloroplast is often more or less coarsely reticulated. Numerous small free plates such as those described by Schmitz (1882) have never been observed during this work, except in the case of a form of *Cladophora* which had been kept indoors in abnormal conditions for several weeks and was obviously in very bad condition. This observation supports those of Brand (1901), who also expressed the opinion that this condition of the chloroplast was not normal.

The small outgrowths described by Wille (1901), projecting from the external surface of the chloroplast in *Rhizoclonium riparium*, have never



TEXT-FIG. 2. *Chaetomorpha*, Kütz. A and B, *Ch. gracilis*, Kütz. Transverse sections. × 610. C, *Ch. Linum*, (Muell.) Kütz. Longitudinal section. × 510. *pp*, protoplasm.

been observed in any of the species examined during this work Nor is it likely that their absence was due to the process of fixation, since quite similar structures have been well preserved in Desmids which were subjected to the same treatment. The chloroplast was found in every species to be closely applied to the interior of the cell-wall and its external surface was always quite smooth (Figs. 1–8; Text-figs. 1 and 2).

Sections revealed the fact that the chloroplast, as has already been stated by Schmitz (1879, 1882), is not always confined to the periphery of the cell,

but frequently penetrates into the interior and ramifies in all directions (Figs. 1, 2, 4, 6, and 7; Text-fig. 1, A, F, and G, Text-fig. 2, B).

Again, the extent of this depends on the individual cell. In the older segments of *Cladophora* and the large segments of *Chaetomorpha Linum*, (Muell.) Kütz., the entire chloroplast is confined to a very thin parietal lining layer, and the interior of the cell is occupied by a large vacuole, being destitute of both protoplasm and chloroplast (Figs. 5 and 8; Text-fig. 2, C).

In the younger segments of *Cladophora*, however, and the rather smaller segments of *Chaetomorpha gracilis*, Kütz., and some forms of *Rhizoclonium hieroglyphicum*, (Kütz) Stockm., which are much richer in cell-contents, the interior of the cell is often traversed by irregular ramifying strands of chloroplast (Figs. 1, 2, 4, 6, and 7; Text-fig. 1, A, F, and G, Text-fig. 2, B).

The pyrenoids are very numerous, particularly in *Chaetomorpha* and *Cladophora*, and are to be found in every part of the chloroplast (Figs. 1–8; Text-figs. 1 and 2). It was noticed in *Rhizoclonium hieroglyphicum* that the pyrenoids in the internal strands of chloroplast were much larger in size than those occurring in the thin parietal lining layer, possibly because they can develop far more freely in this position (Text-fig. 1, F).

When the interior of the segment is filled by these ramifying strands of chloroplast it is often very difficult to distinguish between the chloroplast and the colourless cytoplasm. Of course, when the external parietal sheath of chloroplast is reticulated it is quite easy to demonstrate the colourless cytoplasm filling in the perforations in the chloroplast (Fig. 3). Where the chloroplast is almost continuous over the whole cell-wall, however, colourless cytoplasm is often apparently non-existent. In sections, also, it often appears that every available strand of cytoplasm is occupied by chloroplast and pyrenoids (Figs. 1 and 6). It is sometimes possible, however, to distinguish extremely delicate, nearly colourless, granular strands, destitute of pyrenoids and usually of nuclei also, which doubtless represent the colourless cytoplasm (Text-fig. 2, B); but very frequently the chloroplast is so extensive, and penetrates both the lining layer of cytoplasm and the internal strands traversing the lumen of the cell so completely, that the colourless protoplasm is reduced to an extremely thin film enveloping the chloroplast—so thin that it cannot be detected.

The relation of the nuclei to the chloroplast is rather unusual in these three genera. In other algae where there is a definite chromatophore, the nucleus is usually confined to the colourless cytoplasm, but in *Cladophora* and *Chaetomorpha* particularly the nuclei are nearly always more or less completely immersed in the chloroplast. They are scattered throughout both the parietal lining sheath of chloroplast and also in the internal strands traversing the cell-cavity (Figs. 1, 2, 4–8; Text-figs. 1 and 2). In the parietal layer no definite colourless cytoplasmic sheath can be detected round the nucleus, and in the interior of the cell they usually occur in such close

association with the pyrenoids that here also it is quite impossible to distinguish between definite colourless cytoplasm containing nuclei and chloroplast with pyrenoids (Figs. 1 and 2).

The structure is very suggestive of that described by Timberlake (1901) for *Hydrodictyon*. Timberlake found that in this alga the nuclei and pyrenoids often occurred in such intimate connexion that he was unable to distinguish a definite chromatophore, and so he decided that the chlorophyll must be diffuse and evenly distributed throughout the cytoplasm. The nuclei are nearly always in the same close association with the pyrenoids in the Cladophoraceae, but since it is sometimes possible to distinguish a coarsely reticulated chloroplast lying against the cell-wall with colourless interstices, it is quite obvious that in this case the chlorophyll cannot be diffuse. In these forms it is rather that the chloroplast has become so massive and has invaded the cytoplasm to such an extent that it naturally engulfs the nuclei as well. Moreover, the nuclei seem to prefer this position within the chloroplast rather than the colourless cytoplasm itself, for even in cells where the parietal film of chloroplast is very scanty, and more of the lining layer consists of colourless cytoplasm than is occupied by the chloroplast, the nuclei are still to be found in the chloroplast itself, although there is plenty of room for them in the colourless cytoplasm filling in the interstices (Fig. 3).

Börgesen (1913), although not remarking particularly on the position of the nuclei, similarly figures these bodies apparently within the chromatophores in two members of the Siphonocladiales, *Cladophoropsis membranacea*[1] and *Siphonocladus tropicus*.[2] In another species, however, *Boodlea Siamensis*,[3] the nuclei are figured in the colourless cytoplasm lining the cell-wall, between the chloroplasts, whilst in *Dictyosphaeria favulosa*,[4] *Chamaedoris Peniculum*,[5] and *Ernodesmis verticillata*[6] the nuclei are described as being either near to or underneath the chloroplasts in the cell-plasma. Thus it is quite possible that Börgesen really intended that in the first two species also, the nuclei should be imagined to lie in the protoplasm under the chromatophores, but this point is not made absolutely clear.

There seems to be no doubt, however, that in the species of *Cladophora* and *Chaetomorpha* examined during this investigation most of the nuclei are immersed directly in the chloroplast. It is only very rarely that one meets with a nucleus in the interior of the cell which is not in close association with pyrenoids, and might possibly be quite free from the chloroplast.

The same remarks apply with very slight modification to *Rhizoclonium hieroglyphicum*. In a very narrow form examined, the chloroplast was confined almost entirely to the peripheral parts of the cell, forming a very thin,

[1] p. 47. [2] p. 66. [3] p. 50. [4] p. 34.
[5] p. 58. [6] p. 68.

more or less reticulated parietal film. The nuclei were in this form of very large size in comparison with the small diameter of the cell, and therefore projected considerably from the thin parietal film of chloroplast into the cell-cavity. Thus they were usually not entirely immersed in the chloroplast (Text-fig. 1, C and D).

In a rather thicker form examined the condition approached more nearly that found in certain forms of *Cladophora* (Text-fig. 1, E–G).

The projecting of the nuclei into the cell-cavity is often observed in some forms of *Cladophora* and *Chaetomorpha* in which the lining parietal film of chloroplast is extremely thin (Figs. 4 and 5; Text-fig. 2), but this is far more pronounced in the narrow form of *Rhizoclonium hieroglyphicum* described above.

With regard to the number of nuclei occurring in the segments of *Rhizoclonium hieroglyphicum*, the narrower form very often had only two, although four and eight were also very frequent, whilst in some very well-nourished filaments, the cells of which had very dense cell-contents, the nuclei were often as many as sixteen. In the larger form the nuclei were always more numerous (Text-fig. 1, G), eighteen to twenty-four being commonly found in segments of medium size. In opposition to this it was noticed that in *Cladophora crispata*, (Roth) Kütz., the nuclei in many of the segments having very scanty cell-contents were very reduced in number, sometimes to eight or nine.

These observations tend to support those of Brand (1898), that the number of nuclei depends not on the genus itself but largely on the cubical content of the cell, and also show that the relative abundance of the cyto plasmic contents of the segment also plays some part in this respect.

Both *Rhizoclonium* and *Cladophora* accumulate large quantities of starch during the autumn months of the year, and material was therefore examined in this condition in order to get exact details of its storage. In the external view the parietal film of chloroplast is usually seen to be quite continuous, and its protoplasmic reticulum is very coarse, the cavities between the meshes being occupied by innumerable starch-grains (Text-fig. 1, B).

Thus the whole of the chloroplast between the pyrenoids is densely packed with starch-grains, and these also extend into the films of chloroplast between the nuclei and the cell-wall, so that the nuclei are often pushed farther into the interior of the cell. This is better seen in sections (Fig. 2; Text-fig. 1, A).

The strands of chloroplast traversing the lumen of the cell are similarly packed with starch, and become very distended, occupying even more of the cell-cavity. In this condition no trace of colourless cytoplasm can be detected, and the nuclei are even more completely enveloped by the chloroplast (Figs. 2 and 7; Text-fig. 1, A).

In *Rhizoclonium* the cell becomes nearly solid with pyrenoids and starch,

the vacuoles diminishing as the chloroplast becomes more and more distended (Text-fig. 1, A).

In *Cladophora* it is quite certain that these starch-grains which accumulate in the chloroplast had their origin in the pyrenoids, as explained by Timberlake (1901), since they correspond fairly well with the starch-grains of the starch-sheaths in size and shape, and are often seen grouped in circles round the pyrenoids, having just been thrown off. In *Rhizoclonium*, however, this is not quite so clear, for most of the grains lying free in the chloroplast are somewhat smaller in size than the large and peculiar grains which make up the sheaths of the pyrenoids.

Division of the Nucleus.

Division of the nucleus was observed in *Cladophora glomerata*, var. *simplicior*, Kütz., and also in *Rhizoclonium hieroglyphicum*, and was found to be very similar in both species. The resting nucleus is a more or less spherical body with one or more deeply staining karyosomes (Figs. 9, 23, and 24).

In the particular form of *Cladophora* examined the nucleoli were often particularly numerous, as many as three to five being present in some of the nuclei (Figs. 24 and 25). The nuclear reticulum contains practically no chromatin.

The beginning of division is marked by the gradual disappearance of the nucleoli, the chromatin becoming dispersed through the whole nucleus, finally becoming associated into a spireme which in both species is very long, thin, and convoluted (Figs. 10–12 and 25–28). The contraction of the spireme was particularly clear in the case of *Rhizoclonium* (Figs. 12–14).

The shortened and thickened spireme was later observed to have split into a number of chromosomes which arranged themselves on an equatorial plate (Figs. 15, 29, and 30). The chromosomes are in both cases rod-like structures and are so numerous that it is almost impossible to count them. Division of the chromosomes evidently takes place whilst they are on the equatorial plate, for in the next stage two still very large groups of chromosomes are seen being pulled to opposite poles by the fibres of the nuclear spindle (Figs. 16–18 and 31).

The telophase of the division is characterized by the gradual separation by constriction of the daughter nuclei. This is accomplished by the contraction of the spindle in the region of the equator, some of the fibres being still visible for some considerable time (Figs. 18–20 and 32–34).

Thus the daughter nuclei remain connected by a bundle of fibres which gradually diminishes in thickness and finally disappears altogether, leaving the two nuclei quite isolated (Figs. 21 and 35).

Meanwhile, within the daughter nuclei themselves the chromosomes lose their identity and the chromatin rearranges itself, forming first a rather

indistinct daughter spireme and finally the typical resting nucleus with one or more nucleoli (Figs. 20–2 and 35–7).

Some very peculiar nuclear division figures at the anaphase stage were observed in *Cladophora glomerata*, var. *fasciculata*, (Kütz.) Brand. In these nuclei the migration of the chromosomes to opposite poles was extremely disorderly, the chromosomes following one after the other in an irregular fashion instead of being pulled apart by the fibres of the spindle in two compact masses, whilst the spindle itself was often bent (Figs. 38–40). These peculiar stages were taken from segments in which rapid nuclear division was taking place preparatory to the formation of zoogonidia, but whether this apparently abnormal mitotic figure is general in such cases could not be ascertained.

Summary.

The chloroplast in *Cladophora*, *Chaetomorpha*, and *Rhizoclonium* consists invariably of a parietal film lining the cell-wall and often more or less perforated or reticulated according to the relative abundance of the cell-contents. In most cells with plentiful contents there arise from this lining layer irregular strands which traverse the lumen of the cell, and in many cases are so numerous that they occupy a considerable part of the cell-cavity. In mature cells, or cells with very scanty cell-contents, this internal network of chloroplast may be wanting.

Pyrenoids are very numerous and are scattered in both the peripheral and internal parts of the chloroplast.

Colourless cytoplasm is very difficult to distinguish except where the lining parietal layer of chloroplast is perforated, and the nuclei are almost invariably confined to the chloroplast, not being found as a general rule in the colourless cytoplasm.

In some narrow forms of *Rhizoclonium* the nuclei are of large size in comparison with the small diameter of the cell, and in these the nuclei may not be wholly immersed in the chloroplast, but may often project into the cavity of the cell.

The nuclei in *Rhizoclonium hieroglyphicum* are not so restricted in number as was formerly believed to be the case, since in some thick forms they frequently number twenty-four in a cell.

In the autumn much starch is accumulated and is stored in the form of small grains, which are lodged in the interstices of the protoplasmic reticulum of the chloroplast. As a result of this the chloroplast becomes very distended, and occupies relatively much more of the cell-cavity.

During mitosis the nucleus of *Rhizoclonium* and *Cladophora* is characterized by the formation of a long thin spireme, which gives rise to very numerous chromosomes. After the migration of these to opposite poles of the spindle the daughter nuclei are separated by constriction of the spindle in the region of the equator.

In conclusion, I wish to acknowledge my indebtedness to the late Professor G. S. West, who not only suggested this subject for study, but also gave much help and advice during the investigation.

THE BOTANICAL LABORATORY,
UNIVERSITY OF BIRMINGHAM.

BIBLIOGRAPHY.

BÖRGESEN, F. (1913): The Marine Algae of the Danish West Indies. Pt. I. Chlorophyceae. Copenhagen, 1913.

BORZI, A. (1883): Studi Algologici. Messina, 1883.

BRAND, F. (1895): Ueber drei neue Cladophoraceen aus bayrischen Seen. Hedwigia, Bd. xxxiv, 1895.

——— (1898): Culturversuche mit zwei *Rhizoclonium*-Arten. Botan. Centralbl., Bd. lxxiv, 1898.

——— (1899): *Cladophora*-Studien. Bd. lxxix, 1899.

——— (1901): Ueber einige Verhältnisse des Baues und Wachsthums von *Cladophora*. Botan. Centralblatt Beihefte, Bd. x, Heft 8, 1901.

——— (1902): Die *Cladophora-Aegagropilen* des Süsswassers. Hedwigia, Bd. xli, 1902.

——— (1904 A): Ueber *Cladophora humida* n. sp., *Rhizoclonium lapponicum* n. sp., und deren bostrychoide Verzweigung. Ibid., Bd. xliii, 1904.

(1904 B): Ueber die Anheftung der Cladophoraceae und über verschiedene polynesische Formen dieser Familie. Botan. Centralblatt Beihefte, Bd. xviii, Abt. i, 1904.

——— (1906 A): Ueber *Cladophora crispata* und die Sektion *Aegagropila*. Hedwigia, Bd. xlv. 1906.

(1906 B): Ueber die Faserstruktur der *Cladophora*-Membran. Deutsch. Botan. Gesellsch. Bd. xxiv, Heft 2, 1906.

——— (1908): Ueber Membran, Scheidewände und Gelenke der Algengattung *Cladophora*. Ibid., Bd. xxvi, 1908.

——— (1909 A): Ueber die morphologischen Verhältnisse der *Cladophora*-Basis. Ibid., Bd. xxvii, Heft 6, 1909.

——— (1909 B): Zur Morphologie und Biologie des Grenzgebietes zwischen den Algengattungen *Rhizoclonium* und *Cladophora*. Hedwigia, Bd. xlviii, 1909.

GAY, F. (1891): Recherches sur le développement et la classification de quelques algues vertes. Paris, 1891.

KJELLMANN, F. R. (1898): Zur Organographie und Systematik der *Aegagropilen*. Upsala, 1898.

KÜTZING, F. T. (1843): Phycologia generalis, 1843.

ROTH, A. W. (1797–1806): Catalecta botanica, I–III. Lipsiae, 1797–1806.

SCHMITZ, F. (1879): Beobachtungen über die vielkernigen Zellen der Siphonocladiaceen. Festschrift zur Feier des hundertjährigen Bestehens der Naturforscher-Gesellschaft zu Halle a. S., 1879.

——— (1882): Die Chromatophoren der Algen. Verhandl. des Naturwissensch. Vereins d. preuss. Rheinlande u. Westfalens, 1883.

STOCKMEYER, S. (1890): Ueber die Algengattung *Rhizoclonium*. Verhandl. d. k.-k. zool.-botan. Gesellsch. in Wien, 1890.

STRASSBURGER, E. (1880): Ueber Zellbildung und Zelltheilung im Pflanzenreiche. Jena, 1880.

TIMBERLAKE, F. G. (1901): Starch-formation in *Hydrodictyon utriculatum*. Ann. Bot., vol. xv, 1901.

WILLE, N. (1901): Studien über Chlorophyceen, I–VII. Vid.-selsk. Skrifter, I. Math.-naturw. Klasse, 1900. (Christiania, 1901.)

DESCRIPTION OF PLATE XXVII.

Illustrating Dr. Nellie Carter's paper on the Cytology of the Cladophoraceae.

Figs. 9-40 all × 1423.

Fig. 1. *Cladophora glomerata*, var. *fasciculata*, (Kütz.) Brand. Transverse section of a young segment. × 610.

Fig. 2. *Cl. glomerata*, var. *callicoma*, (Kütz.) Rabenh. Transverse section of a young segment in the autumn condition, the chloroplast distended with starch-grains (*st.*). × 610.

Fig. 3. *Cl. crispata*, (Roth) Kütz. Portion of a young segment. × 510.

Figs. 4 and 5. *Cl. fracta*, Kütz. Transverse sections of young and older segments respectively. × 810.

Fig. 6. *Cl. glomerata*, var. *simplicior*, Kütz. Transverse section of a fairly old segment. × 810.

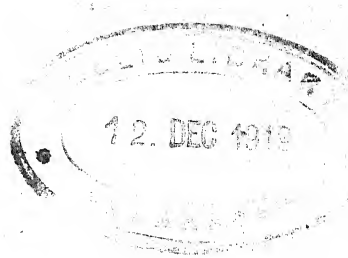
Fig. 7. *Cl. glomerata*, var. *callicoma*, (Kütz.) Rabenh. Longitudinal section of a young segment in the autumn condition (*st.* = starch). × 610.

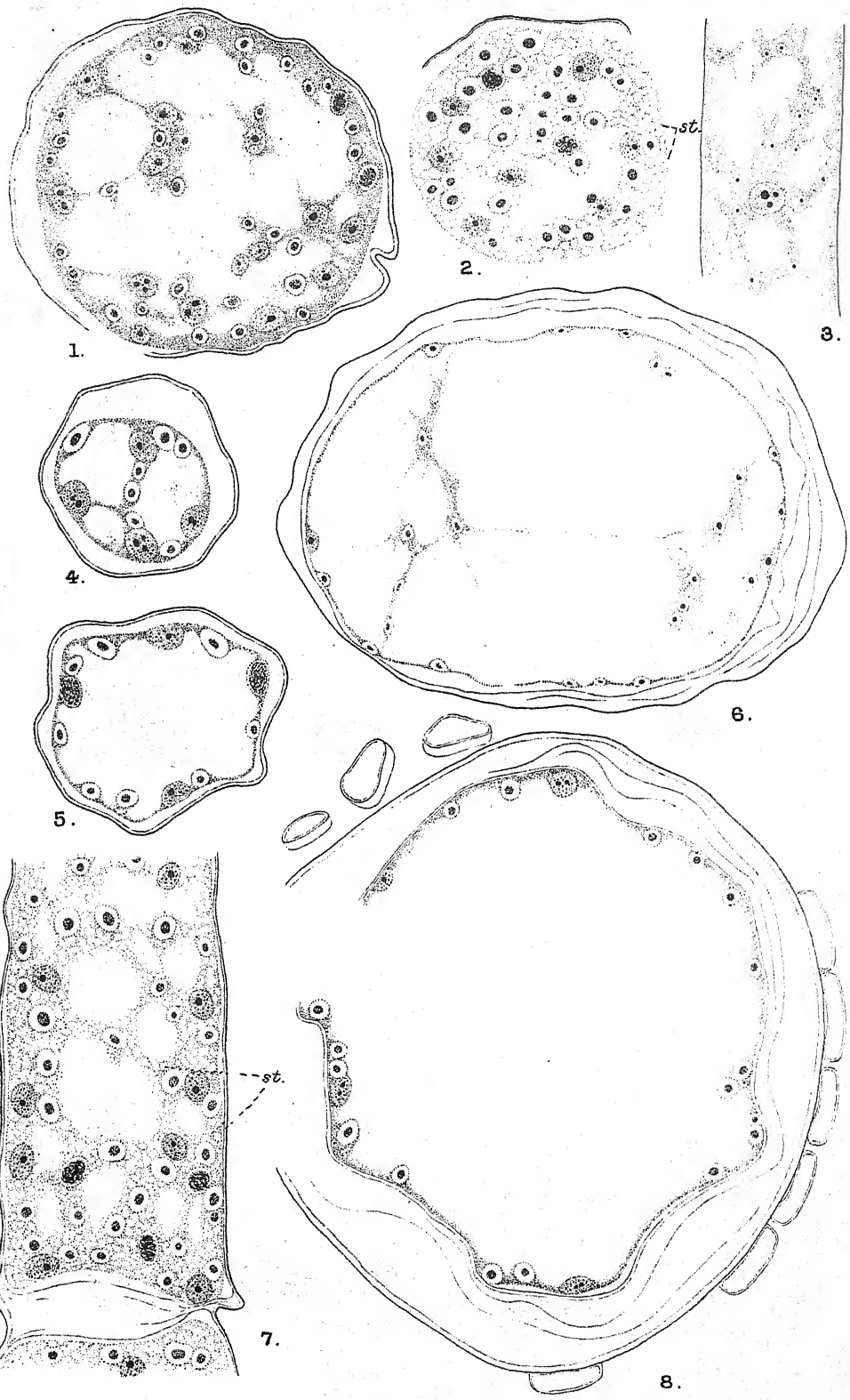
Fig. 8. *Cl. glomerata*, var. *fasciculata*, (Kütz.) Brand. Transverse section of a very old segment with the epiphytic diatom *Cocconeis Pediculus*. × 610.

Figs. 9–22. *Rhizoclonium hieroglyphicum*, (Kütz.) Stockm. Successive stages in the division of the nucleus. Fig. 9, resting nucleus; Figs. 10 and 11, disappearance of the nucleolus; Figs. 12–14, formation of spireme; Fig. 15, appearance of spindle and chromosomes; Figs. 16–18, anaphase; Figs. 19–21, telophase; Fig. 22, daughter nuclei.

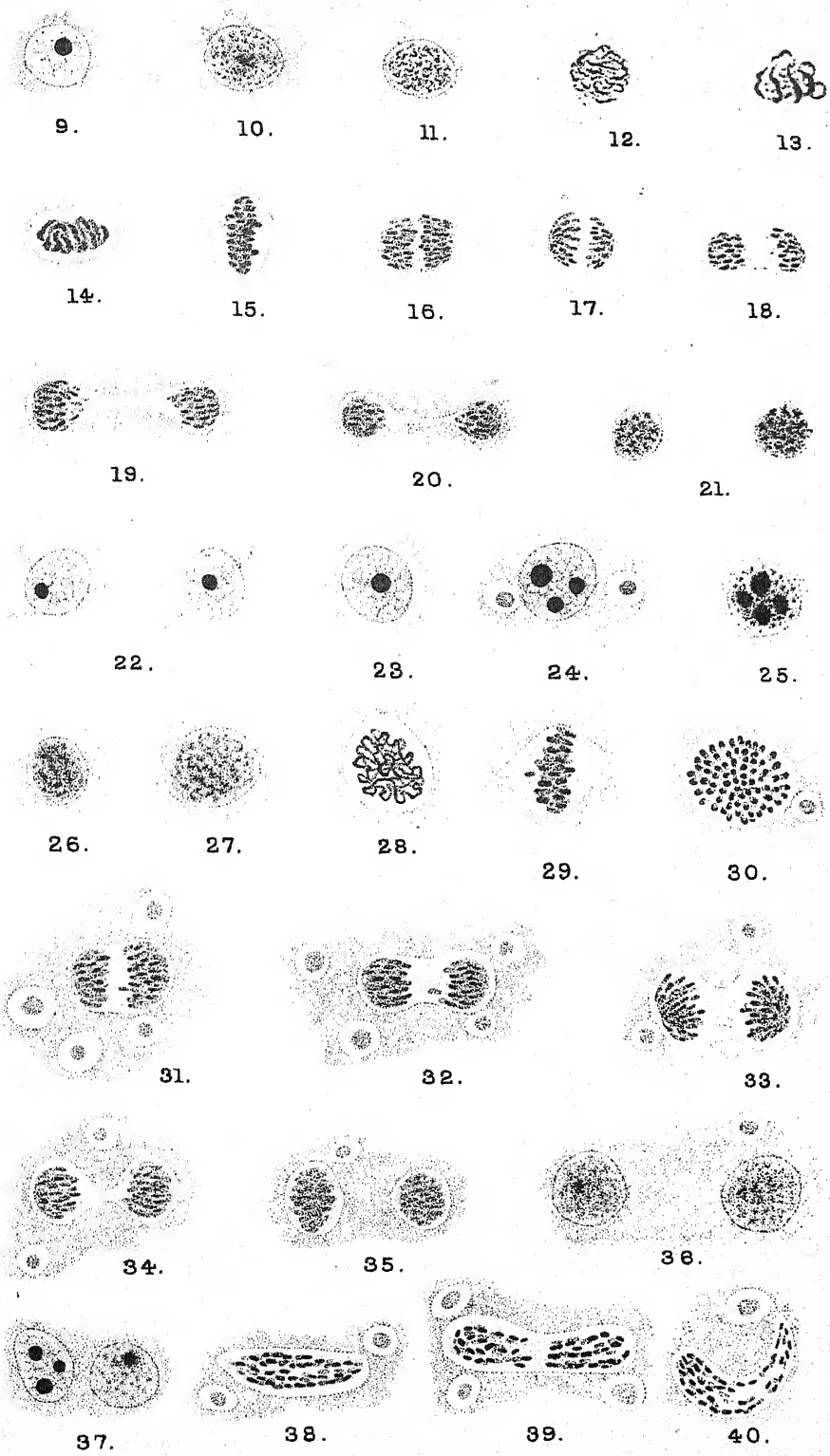
Figs. 23–37. *Cladophora glomerata*, var. *simplicior*, Kütz. Division of the nucleus. Figs. 23 and 24, resting nuclei; Figs. 25–27, disappearance of the nucleoli; Fig. 28, formation of spireme; Fig. 29, formation of chromosomes and spindle; Fig. 30, the spindle in end view; Figs. 31–33, anaphase; Figs. 34-36, telophase; Fig. 37, daughter nuclei, the chromatin in one being not yet entirely collected into a nucleolus.

Figs. 38–40. *Cl. glomerata*, var. *fasciculata*, (Kütz) Brand. Abnormal nuclear division at the anaphase stage.

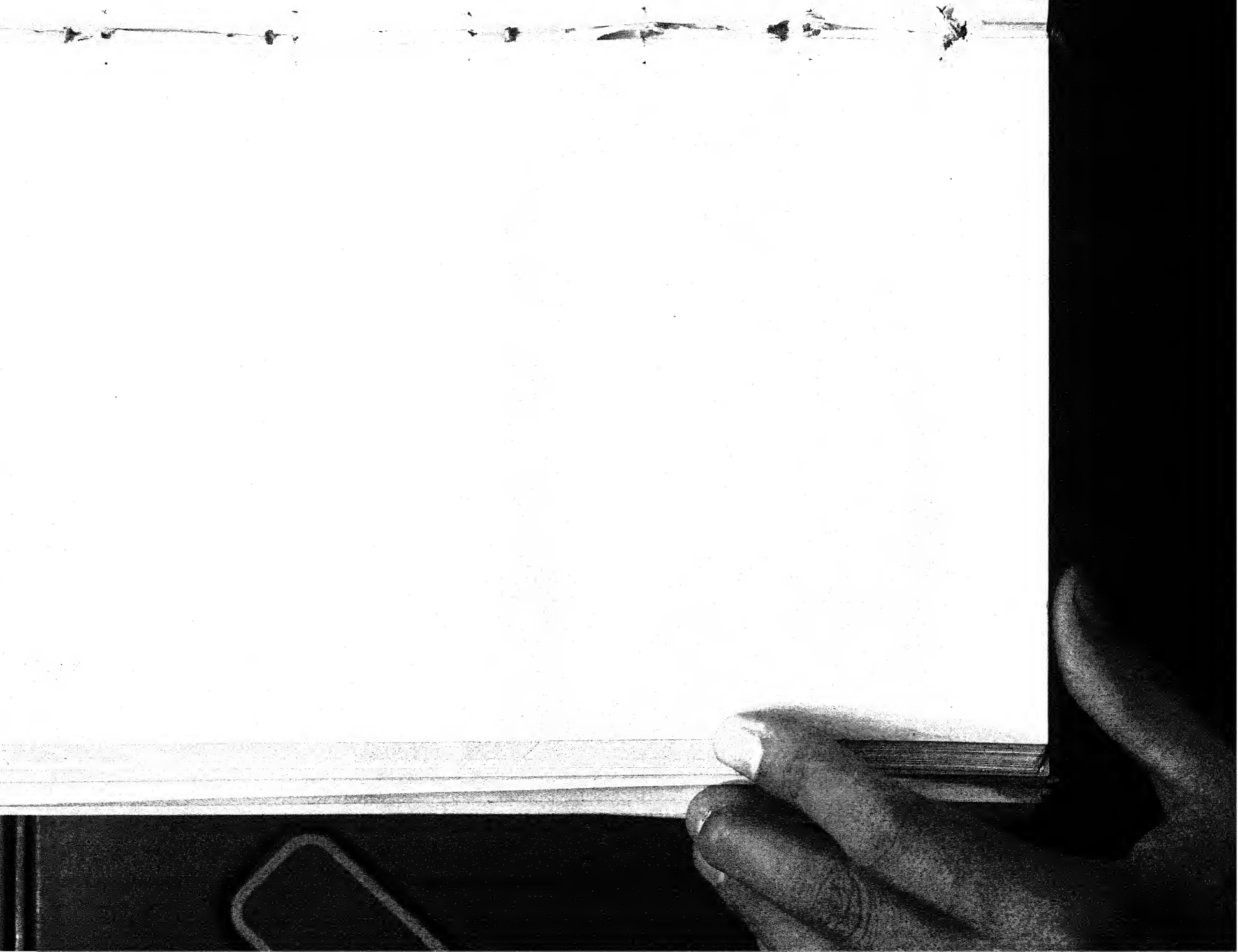

12. DEC 1919



CARTER—CLADOPHORACEAE.


9.
10.
11.
12.
13.
14.
15.
16.
17.
18.
19.
20.
21.
22.
23.
24.
25.
26.
27.
28.
29.
30.
31.
32.
33.
34.
35.
36.
37.
38.
39.
40.

Huth, London.



On the Floras of Certain Islets outlying from Stewart Island (New Zealand).

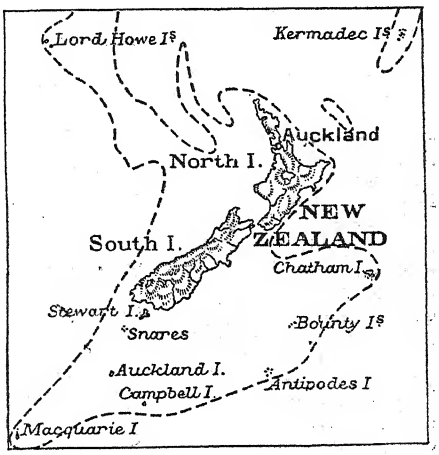
BY

J. C. WILLIS, M.A., Sc.D., F.R.S.,

European Correspondent, Botanic Gardens, Rio de Janeiro.

With one Map in the Text.

AN interesting little paper by Poppelwell on the flora of Long Island, which is separated by one and a half miles of water from the south-west of Stewart Island, has called my attention to further papers on the islets which outlie from Stewart, especially the Breakseas and Solanders, the former close to the east coast of Stewart, the latter about thirty-five miles from the north-west coast and rather nearer to the South Island of New Zealand. These papers[1] are best considered together; they illustrate very clearly the extraordinary applicability of age and area to the New Zealand flora, and suggest a way in which it may be applied even to the flora of Great Britain, where the effects of man's occupation are now so predominant.



New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

The simplest way of dealing with the matter will probably be to first of all arrange these little floras in parallel columns, classified for convenience as in Cheeseman's 'Flora'.

[1] Poppelwell: Notes of a Botanical Excursion to Long Island. Trans. and Proc. N.Z. Inst., xlix, 1917, p. 167.
Notes on the Plant Covering of the Breaksea Islands, l.c., xlviii, 1916, p. 246.
Cockayne: On a Collection of Plants from the Solanders, l.c., xli, 1909, p. 404.

[Annals of Botany, Vol. XXXIII. No. CXXXII. October, 1919.]

TABLE I.

Species in italics are peculiar to one island.

Long Island.	*Breakseas.*	*Solanders.*
CRUCIFERAE		
Cardamine heterophylla	Cardamine heterophylla	—
—	*Lepidium oleraceum*	—
PITTOSPORACEAE		
—	*Pittosporum Colensoi*	—
ROSACEAE		
—	*Rubus australis*	—
SAXIFRAGACEAE		
Weinmannia racemosa	—	—
CRASSULACEAE		
Tillaea moschata	Tillaea moschata	Tillaea moschata
DROSERACEAE		
Drosera spathulata	Drosera spathulata	—
MYRTACEAE		
Leptospermum scoparium	Leptospermum scoparium	—
Metrosideros lucida	Metrosideros lucida	—
ONAGRACEAE		
—	*Fuchsia excorticata*	—
FICOIDEAE		
Mesembryanthemum australe	Mesembryanthemum australe	Mesemb. austr.
Tetragonia trigyna	Tetragonia trigyna	—
UMBELLIFERAE		
Hydrocotyle Novae-Zeelandiae	—	—
Apium prostratum	Apium prostratum	Apium prostratum
Ligusticum intermedium	Ligusticum intermedium	Ligust. intermedium
ARALIACEAE		
Aralia Lyallii	Aralia Lyallii	Aralia Lyallii
Panax Edgerleyi	Panax Edgerleyi	—
Colensoi	—	—
CORNACEAE		
Griselinia littoralis	Griselinia littoralis	—
RUBIACEAE		
Coprosma lucida	Coprosma lucida	—
—	*areolata*	—
foetidissima	foetidissima	—
Colensoi	—	—
Nertera depressa	Nertera depressa	—
COMPOSITAE		
Olearia angustifolia	Olearia angustifolia	—
Colensoi	Colensoi	Olearia Colensoi
—	*Traillii*	—
arborescens	arborescens	—
Celmisia longifolia	—	—
—	*Cotula coronopifolia* (probably introduced)	—
Senecio bellidioides	—	—
—	*Senecio lautus*	—
—	—	*Senecio Stewartiae*
rotundifolius	rotundifolius	rotundifolius
—	*Sonchus littoralis*	—
STYLIDIACEAE		
Donatia Novae-Zeelandiae	—	—
—	*Oreostylidium subulatum*	—
GOODENIACEAE		
—	*Selliera radicans*	—
ERICACEAE		
Gaultheria antipoda	—	—

TABLE I (*continued*).

Long Island.	*Breakseas.*	*Solanders.*
EPACRIDACEAE		
Pentachondra pumila	Pentachondra pumila	—
Styphelia acerosa	Styphelia acerosa	—
empetrifolia	—	—
Dracophyllum longifolium	Dracophyllum longifolium	—
MYRSINACEAE		
Suttonia chathamica	—	—
—	*Rapanea Urvillei*	—
GENTIANACEAE		
—	*Gentiana saxosa*	—
BORAGINACEAE		
Myosotis albida	Myosotis albida	Myosotis albida
SCROPHULARIACEAE		
Veronica elliptica	Veronica elliptica	Veronica elliptica
buxifolia	—	—
PLANTAGINACEAE		
—	*Plantago Raoulii*	—
URTICACEAE		
Urtica australis	—	—
ORCHIDACEAE		
—	*Thelymitra longiflora*	—
—	uniflora	Thelymitra uniflora
—	*Microtis unifolia*	—
—	*Prasophyllum Colensoi*	—
—	*Pterostylis Banksii*	—
—	*australis*	—
—	*Caladenia bifolia*	—
LILIACEAE		
Phormium tenax	Phormium tenax	—
Cookianum	Cookianum	—
Enargea parviflora	—	—
Astelia linearis	—	—
nervosa	—	—
JUNCACEAE		
Luzula campestris	—	Luzula campestris
RESTIONACEAE		
—	*Leptocarpus simplex*	—
CYPERACEAE		
Scirpus aucklandicus	Scirpus aucklandicus	—
nodosus	—	—
—	*Carpha alpina*	—
Gahnia procera	Gahnia procera	—
Oreobolus pectinatus	Oreobolus pectinatus	—
strictus	—	—
Carex lucida	Carex lucida	—
trifida	trifida	Carex trifida
GRAMINEAE		
Hierochloe redolens	Hierochloe redolens	—
Microlaena avenacea	—	—
Poa foliosa	Poa foliosa	Poa foliosa
Astoni	Astoni	Astoni
CONIFERAE		
Podocarpus ferrugineus	—	—
Dacrydium intermedium	—	—
—	*Dacrydium biforme*	—
HYMENOPHYLLACEAE		
Hymenophyllum rufescens	—	—
CYATHEACEAE		
Dicksonia squarrosa	Dicksonia squarrosa	—
Hemitelia Smithii	—	

TABLE I (*continued*).

Long Island.	*Breakseas.*	*Solanders.*
POLYPODIACEAE		
Polystichum vestitum	Polystichum vestitum	—
Asplenium obtusatum	Asplenium obtusatum	Asplenium obtusatum
scleroprium	scleroprium	—
lucidum	lucidum	lucidum
flaccidum	flaccidum	—
bulbiferum	—	—
Blechnum durum	Blechnum durum	Blechnum durum
capense	capense	—
Histiopteris incisa	Histiopteris incisa	Histiopteris incisa
Pteridium esculentum	—	—
Polypodium diversifolium	Polypodium diversifolium	—
GLEICHENIACEAE		
Gleichenia circinata	—	—
SCHIZAEACEAE		
Schizaea fistulosa	—	—
LYCOPODIACEAE		
Lycopodium varium	—	—
ramulosum	Lycopodium ramulosum	—

It is clear, from the fact that these islands are separated from Stewart by some breadth of water, that they must have a very old flora, older on the whole than that of Stewart itself, especially the Solanders, which are over thirty miles away, but are a little nearer to the South Island of New Zealand (so that they might contain species not known in Stewart). We shall therefore expect all their floras to be small, especially that of the Solanders. In actual fact, 73 species are recorded from Long Island, 69 from the Breakseas, and 19 from the Solanders.

One will expect, just as in the more extended case of the Kermadecs, Chathams, and Aucklands, with which this may be compared, that much of the floras will be the same in all the islands. If we take the 19 species of the Solanders, we find in fact that 16 of them also occur both in the Breakseas (on the *other* side of Stewart) and in Long Island, one occurs in Long Island only, and one in the Breakseas only. These two last quite probably occur in both these islands, but have not yet been recorded, and there remains only *Senecio Stewartiae*, which is also recorded for Herekopere Island in Foveaux Strait (as near the Solanders as Stewart itself) and the Snares.

Long and Breaksea islands have much larger floras, and we find on comparison that besides the 16 already mentioned which they have also in common with the Solanders, they have 29 in common between themselves only, making 45 in all. Long Island has 27 species not recorded from the Breakseas, and the Breakseas 23 not recorded from Long Island. These are printed in italics above. Glancing at the lists, it is fairly safe to say that about a dozen at least of those given for the Breakseas only, e. g. the orchids, ought certainly to be found also in Long Island, if it were examined at a different period of the year. On the whole, the resemblances between the floras of these three island groups are very striking. Poppelwell notes these resemblances, but puts them down to similarity of conditions, a cause which

in the light thrown upon geographical distribution by age and area can no longer be accepted as sufficient to account for such phenomena.

Just as we found the flora of Stewart, as older, to be composed of the larger (in general, older) families and genera of New Zealand proper, so here we shall expect the flora of these islands to be composed of the larger families and genera of the Stewart flora, and that of the Solanders especially so. Testing this we find that of the New Zealand families 22 are above the average in size (in New Zealand) and 69 below. Of the former 21 (95 per cent.) occur in Stewart, of the latter only 39 (56 per cent.) Of the Stewart families 15 are above the average (in Stewart) and 45 below. Of the former 13 (86 per cent.) occur in the islets, of the latter 17 (37 per cent.). Of the islet families 9 are above the average and 21 below. Of the former 6 (66 per cent.) occur in the Solanders and of the latter 4 (19 per cent.) only. It is thus clear that on the average a family is represented everywhere in proportion to its size in the neighbouring country. We may put this in another way, thus. The average size in New Zealand and the surrounding islands of a family occurring there (91 fams., 1,392 species) is 15 species. The average size in New Zealand of families occurring in Stewart is 21 species, or much higher. The average size in New Zealand of the families that occur in the islets now under consideration is 35 species (30 fams., 1,059 species). And finally, the average size in New Zealand of the families occurring in the Solanders (11 fams., 765 species) is 69 species. The figures thus form a progressive series, showing clearly that on the whole the larger in New Zealand a family is, the greater in the New Zealand area is its range.

We shall further expect that, as usual, there will be more families in proportion to genera, and more genera in proportion to species, the farther out we go from the centre of New Zealand.

TABLE II.

	Fams.	*Gen.*	*Spp.*	*Gen. per fam.*	*Spp. per gen.*
New Zealand	91	329	1392	3·6	4·2
Stewart	60	169	383	2·8	2·2
Islets	30	56	98	1·8	1·7
Solanders	11	13	15	1·1	1·1

The prediction is fully borne out.

As one goes outward from New Zealand in this way, the plants will on the average become steadily older, so that one will expect to find the proportion in common with the outlying islands (Kermadecs, Chathams, Aucklands), which also have old floras, steadily increasing. Testing this gives

TABLE III.

Occur in		*Each K., Ch., or Au.*	%
New Zealand,	1301	199	15
Stewart,	383	153	40
Islets,	98	52	53
Solanders,	15	9	60

Again a steadily increasing percentage, bearing out the prediction.

Further, one will expect the proportion of wides, which on the whole are older, to increase relatively to that of endemics as one goes outwards from New Zealand to the Solanders.

TABLE IV.

Occur in	*Wides.*	*Endemic (N.Z. and Isl.).*
New Zealand	301 or 23 %	1000
Stewart	129 or 34 %	240
Islets	27 or 35 %	49
Solanders	6 or 40 %	9

If the ferns be included, the result is more clearly marked.

It is thus clear that for restricted areas like New Zealand and its neighbouring islands age and area can be relied upon to explain the general composition of any of the floras that occur; and in our next paper we shall go somewhat farther afield, endeavouring to trace the invasions of New Zealand from Indo-Malaya.

Some Observations on the Tuber of Phylloglossum.

BY

T. G. B. OSBORN.

With Plate XXVIII and forty-three Figures in the Text.

WHEN, towards the end of the growing season 1917, the opportunity offered to study *Phylloglossum Drummondii* in the field near Adelaide, it was felt that some observations of interest might be made upon the ecology of the plant, with special reference to the behaviour of the tuber as an organ of perennation. The scope of the inquiry was unexpectedly extended by the discovery that accidentally damaged or detached leaves might themselves form new tubers as they lay upon the soil near naturally growing plants. A series of observations was therefore made during the growing season (May to October) 1918, the results of which are also given here. Without wishing at this stage to reopen a discussion as to the morphology of *Phylloglossum Drummondii*, it is felt that the facts now presented throw some light on the nature of the tuber that is of importance in considering its morphology.

Field Observations on Phylloglossum.

The external morphology of *Phylloglossum* has been so many times described that a further account might be deemed superfluous. In the present paper, the tuber, formed the previous season, from which arise the stem, leaves, roots, and strobilus (should one be produced), and which is consequently in process of exhaustion, is termed the 'current tuber'.[1]

The term 'old tuber' is restricted to those of previous years, which may be found in the soil beside growing plants.[2] These may throw a light upon the past history of the plant near which they are found.

On the Depth of Tuber Formation—Descriptive.

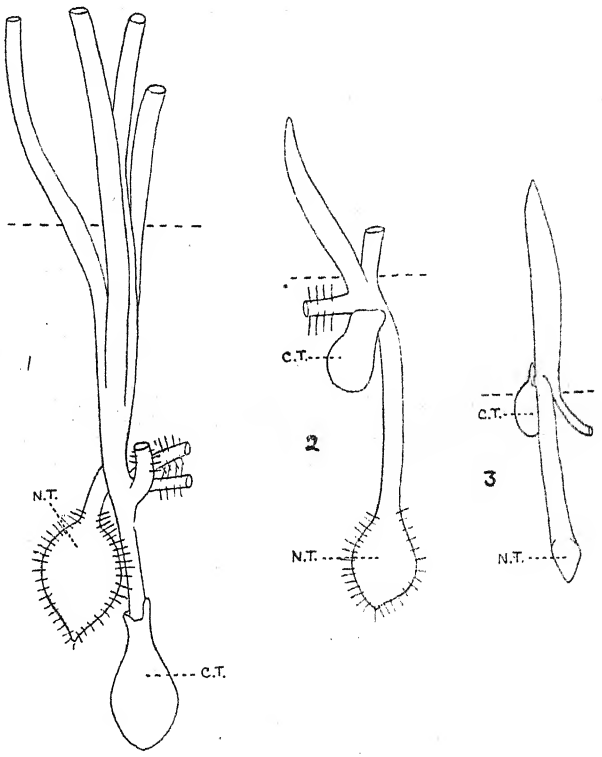
On examining any large number of plants of *Phylloglossum* collected towards the end of their growing season, the two tubers are usually observed side by side at about the same depth below the ground-level. Unlike the

[1] Wernham, H. F. (1910): Ann. Bot., xxiv, p. 335.

[2] Thomas, A. P. W. (1902): Proc. Roy. Soc., London, lxix, p. 288.

perennating organs of many geophytes, these two structures, products of successive seasons' activities, are not directly connected. The new tuber is linked to the current one only by its stalk, which is given off from the stem of the plant usually some distance above the current tuber. Clearly, then, the new tuber is produced sunken in the soil because of the downward growth of its stalk. Whilst the growth is usually of such an amount as to cause the two tubers to lie side by side, this is by no means always the case.

Not infrequently plants are found in the condition shown in Text-fig. 1. Here the new tuber is produced at the end of a relatively short stalk, and is



TEXT-FIG. 1. Four-leaved sterile plant showing current tuber buried about 18 mm. New tuber formed on short stalk at shallower depth (Oct. 1917). $\times 3\frac{1}{2}$.

TEXT-FIG. 2. Two-leaved sterile plant with current tuber 4 mm. below ground-level. New tuber formed on long stalk. Sunken about 11 mm. (Oct. 1917). $\times 3\frac{1}{2}$.

TEXT-FIG. 3. Single-leaved plant with current tuber partially exposed. New tuber as yet scarcely swollen, but sunken to 7 mm. (Sept. 14, 1918). $\times 3\frac{1}{2}$.

thus placed vertically in the soil several millimetres nearer the surface than the current tuber.

Or again, specimens may be found in which the current tuber lies just below the surface of the soil (Text-fig. 2) or even partially exposed (Text-fig. 3). Here the new tuber is produced at the end of a relatively long

stalk, hence it may be buried in the soil two or three times its own length deeper than the current tuber.[1]

The examination of any considerable number of plants collected at one time from the same locality shows that the depth at which the new tuber is formed in the soil in relation to ground-level is not a matter of chance, but is the result of definite growth of the plant producing it. If, for any reason, the current tuber is buried by deposition of more soil above it, the burial is compensated for by the development of a short stalk bearing the new tuber. Conversely, if, by removal of surface soil, the current tuber is brought near to the surface, the new tuber is sunken to a greater depth.

There are two sets of climatic conditions that tend to produce an alteration of the ground-level in the area where *Phylloglossum* has been studied near Adelaide.[2] In the wet season (April to October) or during periods of heavy precipitation, which occasionally occur in the summer, there is, owing to local irregularities in the ground, a flow of water over the surface in many places. This causes a removal of soil from some areas and its consequent deposition over others. Since the ground over the greater part of the area is almost flat, the amount of change in level is slight; but very slight changes have a considerable effect on such a minute plant.

On the other hand, during the dry season, when for weeks together the surface layers of the soil are desiccated, atmospheric denudation causes similar slight changes in ground-level. Such variations may not exceed a few millimetres rise or fall, but they influence to a marked degree a plant whose total length from tip of leaf to base of tuber may be less than 3 cm. and the optimum depth for whose tuber lies between 10 and 12 mm.

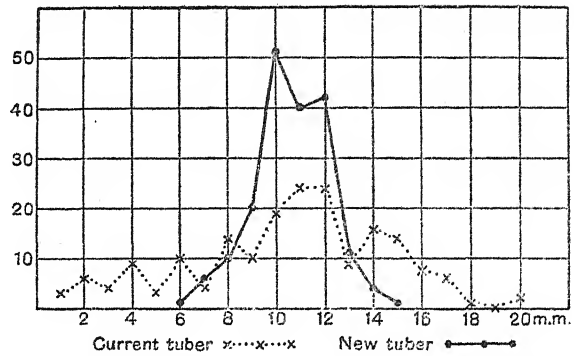
On the Depth of Tuber Formation—Statistical.

In order to gain a clear idea of the depths at which the tubers of a number of *Phylloglossum* plants taken from any area may occur, three typical groups of plants were selected in October (i. e. near the close of the growing season) and a sod of soil some 10 cm. square cut from each. The three sods were carefully removed to the laboratory, where every plant was dissected out. Two measurements were then made upon each plant, viz. the maximum depths of the current and new tubers. The points of measurement selected were the ground-level (usually sharply defined by the development of chlorophyll) and the deepest point of the tuber. It was found desirable to include the length of the tuber in the measurement in order to avoid minus quantities when the tuber was partly exposed, and also because the distal end of the tuber is well defined while the proximal end passes gradually into the stalk.

[1] Bertrand's figure of *Phylloglossum* reproduced in Campbell, D. H., Mosses and Ferns, p. 501, Fig. 290 A, shows this condition, but to a less extreme degree than either of the plants figured above. I regret I have not been able to consult a copy of Bertrand's memoir in Australia.

[2] Osborn, T. G. B. (1918): Trans. Roy. Soc., S. Aus., xlii, p. 7.

In all 186 plants were obtained from the 300 sq. cm. (approx.) of soil. The figures obtained are surprising in the close way they agree with the result that field observations had indicated.



TEXT-FIG. 4. Graph showing variation in depth of current and new tubers on 186 plants collected from (approx.) 300 sq. cm. soil (Oct. 1917).

Phylloglossum Drummondii, 186 plants from 300 sq. cm. soil.

Depth of tubers in mm.	*Current.*	*New.*
Average	10·5	10·6
Minimum	1	6
Maximum	20	15
Mean	10·5	10·5

The graphic representation of the results shown in Text-fig. 4 is specially interesting. The depths of the current tubers give a very irregular flat curve with the mode at 11·5 mm. but with secondary maxima at 8 mm. and 14 mm. The depths of the new tubers, on the other hand, give an almost symmetrical curve about the mode at 10 mm. The curve, moreover, is a steep one, over 80 per cent. of the individuals falling between 9 mm. and 12 mm.

On the Direction of Growth by the New Tuber.

At this stage it may be well to record an observation that bears on the factors determining the direction of growth by the new tuber. The new tuber has always been found upright in the soil, its long axis being vertical. This suggests that gravity is the determining factor in the direction of growth. In compensating for excessive depth at which the current tuber may have been buried, the new tuber never grows erect or even horizontally. A shallower depth is attained merely by the slight amount of elongation made by the new tuber stalk. The flexure made by this stalk is always sharply downwards. Since the point upon the stem from which the new tuber arises is apparently determined by morphological reasons (this is certainly so in the case of sterile plants),[1] it may happen that when

[1] Bower, F. O. (1886): Phil. Trans., clxxvi, p. 669.

the current tuber is very deeply sunken the plant cannot in one season adjust the new tuber to the normal or average depth. Thus two plants having the current tuber buried to 20 mm. produced their new tubers at 15 mm. and 14 mm. respectively. Assuming no change occurred in the ground-level, the new tubers of the ensuing season would also be on short stalks; thus two, or even three, seasons might pass before the average depth was established.

An example of the influence of gravity upon the direction of growth of the new tuber stalk was furnished by a plant growing in the laboratory. In cutting a sod of soil, to be kept moist under a bell-glass for experimental purposes, the trowel accidentally exposed, without damaging, the new tuber stalk of a five-leaved sterile plant. The stalk at that date (July 21, 1918) was still short, the apex unswollen. It continued to grow, exposed to light, descending vertically through 19 mm. before it buried itself in the silt at the bottom of the dish (October 21, 1918). The growth here was clearly geotropic. Light had no influence upon it, for the stalk, though it developed chlorophyll throughout its whole exposed surface, made no phototropic response. This observation is of some importance when considering the behaviour of tubers produced by detached leaves.

On the Rate of Development of Phylloglossum in Successive Seasons.

The sporeling of *Phylloglossum* described by Thomas[1] is single leaved and rootless[2] in the first season of its growth. The number of leaves may be assumed to increase from year to year as successive seasons' activities allow more starch to accumulate in the new tubers.[3] Thomas records that the further development of the sporophyte appears to be slow. 'In many cases the plant comes up a second and third year with only a single leaf.' This he determined by carefully dissecting plants from the soil, and so finding the remains of former years' tubers and roots.[4]

After a little practice it was found possible to remove the remains of the old tubers, together with the plant to which they belonged, without disturbing their relative position one to the other. Text-fig. 5 shows a single-leaved plant collected about a month after the first winter rains. The root is hardly developed and no new tuber is visible at this date, but, in addition to the current tuber, the decaying remains of the tubers formed

[1] Loc. cit., p. 288.

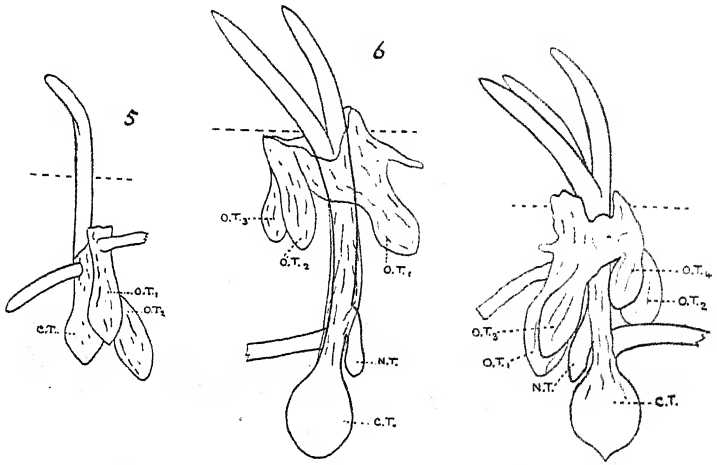
[2] Sampson, K. (1916): Ann. Bot., xxx, p. 605. The sporeling described by Miss Sampson was also single leaved, but had a slender root.

[3] The number of leaves in any season, of course, is not determined until after germination of the tuber. Bower, F. O. (1886): loc. cit., p. 667.

[4] Loc. cit., p. 288.

in two previous years are visible. Such a single-leaved plant is entering on at least the fourth season of its growth.

Text-figs. 6 and 7 show plants of two and three leaves respectively. In Text-fig. 6 three old tuber coats are to be seen. These lie just below the surface of the soil, the current tuber being several millimetres deeper. The past history of this plant, as shown by its old tuber coats, affords a further example of the depth adjustment referred to above. The plant was collected July 1918; the current tuber, therefore, had been formed in the 1917 growing season. That year, the then current tuber, now O.T.$_1$, lay close below the surface of the soil, so that the plant produced its new tuber, C.T. in the figure, at the end of an elongate stalk. Evidently the



TEXT-FIG. 5. Single-leaved plant with current tuber C.T. and two old season's coats O.T.$_1$ and O.T.$_2$. This plant is entering on the fourth season of growth. At this date the root is short and no new tuber visible (June 13, 1918). $\times 3\frac{1}{2}$.

TEXT-FIG. 6. Two-leaved plant with current tuber C.T. at 11 mm. depth, new tuber N.T. just forming. Coats of three previous seasons' tubers (O.T.$_1$, O.T.$_2$, O.T.$_3$) are just below ground-level, showing depth adjustment in 1917 (July 11, 1918). $\times 3\frac{1}{2}$.

TEXT-FIG. 7. Three-leaved plant with current tuber C.T. at 9 mm. depth. Old tuber coats (O.T., &c.) of four previous seasons at shallower depth (July 11, 1918). $\times 3\frac{1}{2}$.

lowering of the ground-level occurred some time subsequent to the 1916 growing season, for lying at the same depth as O.T.$_1$ are O.T.$_2$ and O.T.$_3$, tubers of two previous seasons.

Around another plant no less than four old tuber coats were found (Text-fig. 7), all lying above the current tuber, again demonstrating the burial of the perennating organ to a safe depth. This plant, though it possesses but three leaves, was entering on at least the sixth year of its growth.

Such examples illustrate Thomas's observation on the slow rate at which the sporophyte develops. They also show to a slight extent the precarious existence led by the *Phylloglossum* in some areas, as it seesaws

in the ground, threatened by burial or desiccation, as a result of the edaphic and climatic conditions under which it grows. Development is by no means always a steady progress in which, through successive seasons, the tuber gradually attains a maximum size. In cases where considerable depth adjustment has to be made, or where the early onset of the dry season brings vegetation to a standstill,[1] the new tuber formed may be smaller in size than that from which the plant producing it was derived.

Production of Tubers by Leaves.

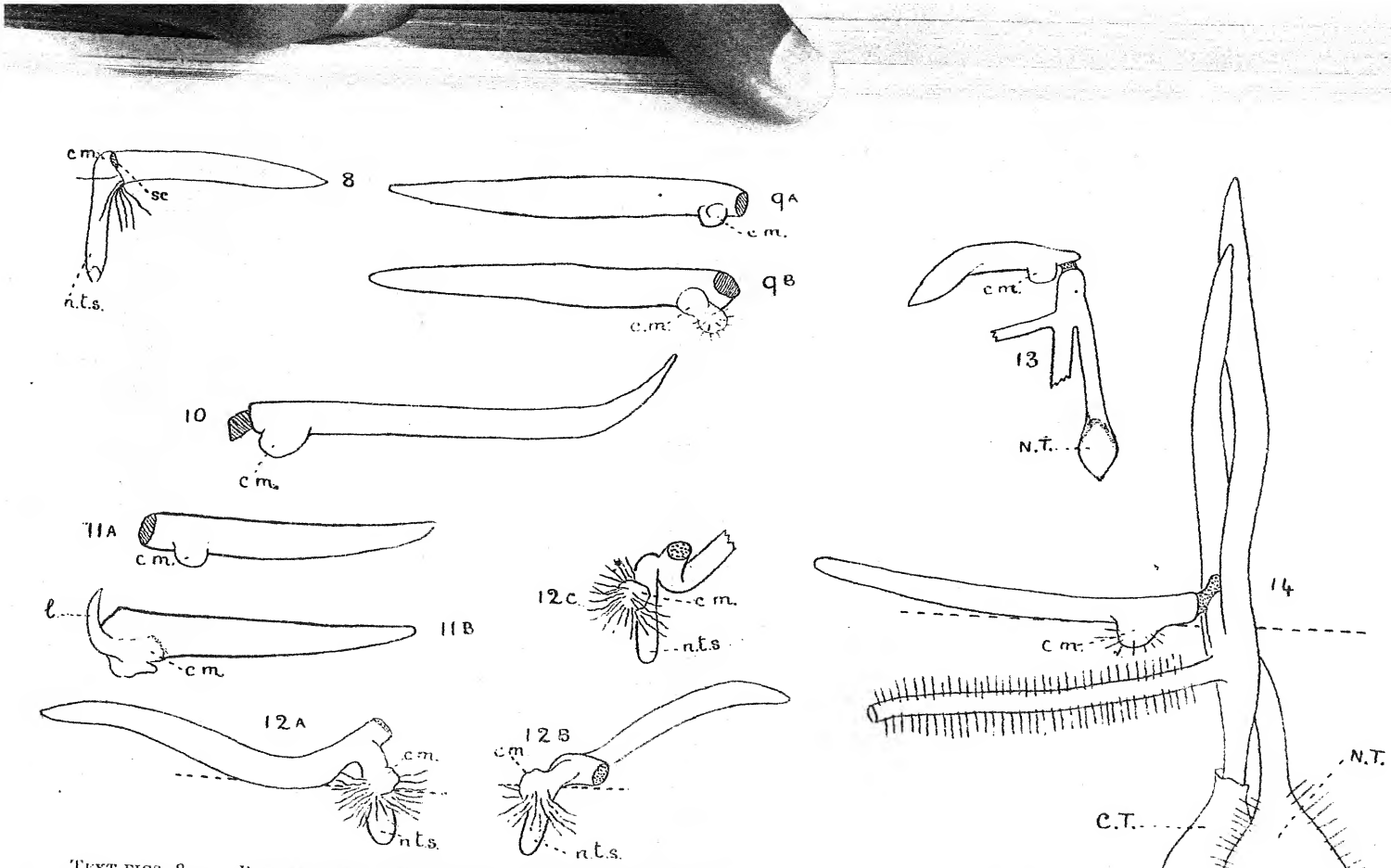
Field Observations 1917.

In 1917, when dissecting plants of *Phylloglossum* from the soil for the statistical investigation above, a specimen was found which at first could not be interpreted. A small green leaf-like structure was found lying in the soil (Text-fig. 8), pointed at one end, the other terminating bluntly, smooth, and rounded. When removed from the soil it was found that this blunt end had a colourless cylindrical prolongation, nearly as long as the aerial portion of the structure, descending vertically into the ground. Its apex showed a minute white spot, and resembled the apices of young tuber stalks of *Phylloglossum*. In the angle made by the two portions a few colourless rhizoids were developed.

When searching for similar structures the leaf shown in Text-fig. 9 A was found. This was obviously a detached leaf of *Phylloglossum*, but near its proximal end a slight swelling of adventitious tissue had developed on the side next the soil. Such a pad of adventitious tissue, which in the following pages will be termed the 'cell mass' for convenience of reference, has been invariably observed as a preliminary development in growth from detached leaves of *Phylloglossum*. Further search yielded two similar leaves, Text-figs. 10 and 11 A, also a third, in which, however, the cell mass was better developed (Text-figs. 12 A, B, and C). This last leaf was somewhat curved and lay upon the soil with its proximal end slightly elevated. It had produced laterally, from the convex side, a cell mass about 2 mm. long; this was swollen irregularly at the farther end and was partly embedded in the soil. The cell mass was green where exposed to the light, while from the lower side of the swollen end rhizoids were produced. From the under surface of this swelling a colourless, almost cylindrical process descended into the soil.

It now became apparent that detached leaves of *Phylloglossum* were capable of producing, after more or less adventitious growth resulting in a cell mass, a structure resembling the stalk of a tuber, such as the plant normally forms each growing season. The structure shown in Text-fig. 8

[1] See p. 508, for rainfall record at Belair, S.A., near which township *Phylloglossum* has been studied. In 1915 only 1·37 in. of rain fell during the last three months of the year.



TEXT-FIGS. 8–14. Regenerating leaves collected in field (Oct. 1917). Lettering throughout: *c.m.* = cell mass, *n.t.s.* = new tuber stalk. Except where otherwise stated, viewed from side. All × 5½. Fig. 8. Detached leaf lying horizontally on soil showing regeneration. A stalk's minute tuber has formed from the cell mass developed at proximal end. The scar (*sc.*) is almost obliterated. Fig. 9 A. Leaf as collected. Fig. 9 B. Same after seven weeks on moist soil. Fig. 11 A. Leaf as collected. Fig. 11 B. Same after seven weeks on moist soil. Note development of 'leaflet' (*l*). Fig. 12 A. Leaf with unusually large cell mass formed at ground-level, also stalk with growing-point beginning to elongate. Figs. 12 B and C. Same viewed from proximal end. Note expansion of cell mass at ground-level, also its development from convex side of curved leaf. Fig. 13. Single-leaved plant forming adventitious cell mass on leaf above injury, also new tuber; current tuber not shown. Fig. 14. Three-leaved plant with cell mass forming from proximal end of broken leaf.

was evidently such a leaf on which the cell mass was small in size, but had almost obliterated the scar formed when the leaf was detached. This scar, somewhat exaggerated in the figure, was so minute that it was not recognized when first examining the leaf.

Two further plants of interest were found in the field that season. One, a single-leaved plant, had suffered an injury about 1 mm. above the soil. The leaf was broken through and lay along the soil, but was not actually detached. The wounded surface had healed over, and a new tuber was forming normally (Text-fig. 13). The broken leaf, however, had not died, but was turgid and green beyond the zone of injured tissue. It had, moreover, begun to form a cell mass upon the lower surface. The second plant was a three-leaved one, one leaf of which had been broken and lay along the soil. Again the wound had healed on both sides of the injury, and the damaged leaf had developed a cell mass near to the proximal end, from the surface of which several rhizoids arose (Text-fig. 14).

The growing season for *Phylloglossum* was almost over in 1917 when these observations were made, so that experimental work was impossible. The two leaves shown in Text-figs. 9 A and 11 A, however, were laid upon soil from the locality in which they had been found, placed in a Petri dish, and kept moist in the laboratory. After about seven weeks it became necessary to stop the experiment as the leaves were beginning to decay. That shown in Text-fig. 9 A had developed a further swelling, irregularly spherical, closely connected with the first (Text-fig. 9 B). The new swelling was opaque, white, and bore short rhizoids. The other leaf (Text-fig. 11 A) showed an unexpected development. No stalk-like structure had formed, but the original cell mass was considerably enlarged (Text-fig. 11 B), and a minute green leaf-like scale (*l*) had developed.

Experimental Development, 1918.

In 1918 five series of experiments upon the production of tubers by detached leaves were carried out in the laboratory, the results of which are here given in tabular form, and are further discussed below.

Series Letter.	*Date Expt. started.*	*No. of Leaves.*	*No. formed single tuber.*	*No. formed single cell mass.*	*No. formed several cell masses.*	*No. died off without apparent growth.*
A.	13. VI	8	1	(1)	6	—
B.	11. VII	8	7	1*	—	—
C.	21. VII	10	5† (+3)	(1)	1§	—
D.	21. VII	10	1	1* (+2)	1	5
E.	13. IX	12	—	5	—	7
		48	14 (+3)	7 (+4)	8	12

† In one of these cases two tubers formed (Fig. 18 D).

* Leaf died off before experiment finished.

§ Developed two 'leaflets' only.

The numbers in brackets refer to leaves fixed for microtoming before fully developed.

Experimental work was confined to the laboratory for convenience of examination, and because of the uncertainty of the season. The 1917 growing season was a wet one,[1] especially during the critical months September–October, when most active growth in tuber-production occurs. In 1916 also there had been more than average rainfall, but there was no reason to expect a continuance of the conditions in 1918, as indeed proved to be the case. In the different experiments the aim was usually not so much to reproduce the conditions of humidity that obtain in the field, as to maintain a constant moisture. It is not suggested that an atmosphere so near saturation would be maintained in the field for long together, though in some years, e.g. 1916, a high rainfall occurred during the last three months of the year, and conditions might remain favourable to tuber production over considerable periods.

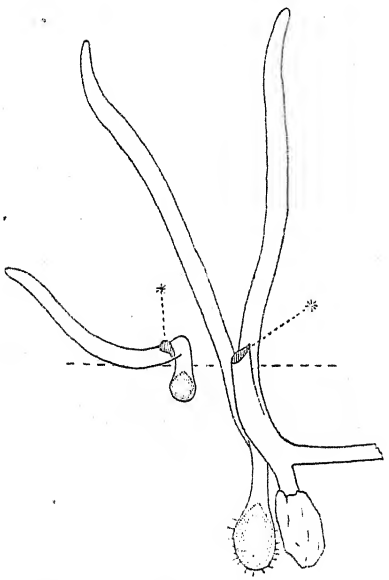


FIG. 15. Three-leaved plant grown in laboratory. One leaf, injured July 17, 1918, has developed an adventitious tuber. *Marks the wounded surfaces which are contiguous in specimen (Nov. 10, 1918). ×3½.

In each experiment freshly detached leaves were laid on soil from the locality, kept moist and covered by glass. In the first experiment the soil was lightly tamped down in a Petri dish, but in the subsequent series a sod several centimetres square in which *Phylloglossum* plants were growing was used. This method was more successful, as will be seen from Series B and C, in which 8 and 10 leaves gave respectively 7 and 8 positive results, but the first method gave a valuable series of abnormalities, discussed later.

In addition to the five series of experiments, odd leaves were injured but not detached from plants kept in the laboratory, the object being to repeat the conditions under which such plants as those shown in Text-figs. 13 and 14 grew. Text-fig. 15 shows the result of such an experiment. One of the three leaves of a plant growing undisturbed in a sod of soil was severely injured but not detached. The plant was dug up and sketched after nearly four months' growth. It had formed a new tuber in the normal way, and the injured leaf, too, had produced a tuber sunken on a short dropper below the surface of the soil. Text-fig. 15 should be compared with Text-fig. 14, which shows a plant collected in the field. The results are closely similar, except that in the experiment the leaf-borne tuber had

[1] See p. 508 below.

almost reached maturity, while in Text-fig. 14 the cell-mass stage only had been attained by the injured leaf.

An attempt to effect regeneration from portions of cone peduncles gave a negative result. The fragments, about 1 cm. long, were laid (July 21) on the same sod of soil as the leaves of Series C. They remained green and turgid for over two months, but early in October began to look unhealthy. On October 21 one only was noticed to have formed a minute swelling near the middle on the upper side, but this peduncle fragment, like the others, had completely collapsed by the end of the month. It is remarkable, as will be seen below, for what length of time a broken fragment of *Phylloglossum* will remain green and apparently healthy on damp soil. On the other hand, once decay sets in it is very rapid, and in a day or two, since there is no hard tissue in the plant, little but the cuticle is left.

(i) *Series B.*

It will be convenient to consider this series first in some degree of detail, since it gave a high percentage of positive results.

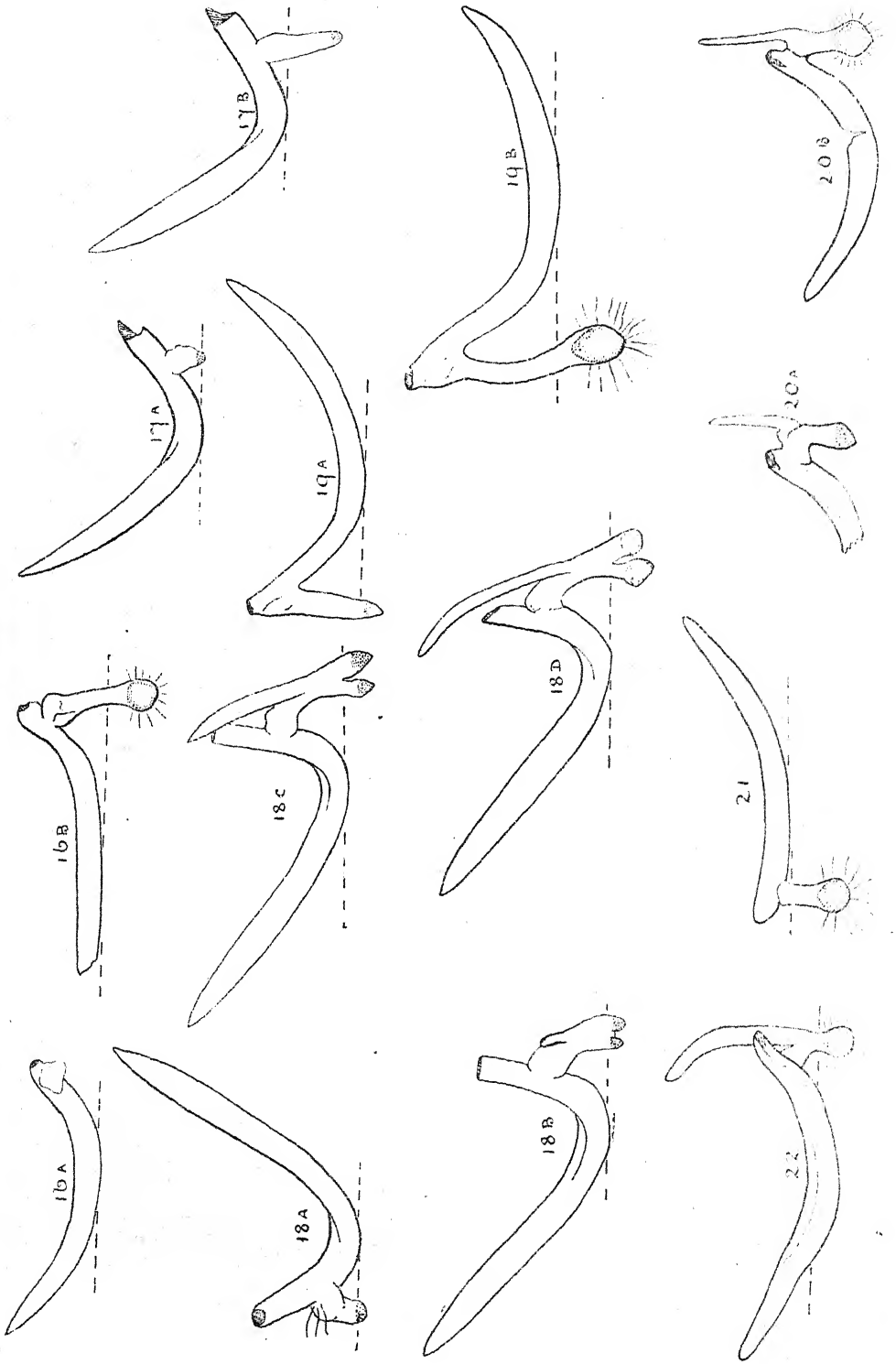
On July 11, 1918, a sod of soil containing many *Phylloglossum* plants was brought into the laboratory and placed in a glass dish. Eight leaves were broken off various well-developed plants growing in it and laid on the soil. The dish was covered and placed in the strong diffuse light of a south window.

During the first month no growth was apparent; the leaves remained turgid and green, the only visible change being a curving of some of the leaves in the vertical plane. This curving, however, was much less apparent than in some of the other series, especially A.

I was unavoidably absent from the laboratory for the fortnight August 15 to 31. Before leaving, the leaves of this and the other series were examined under a hand-lens and the note made that none showed any growth. It was feared that all the experiments were failures (Series A had then been running for two months without apparent result). However, every leaf appeared healthy, turgid, and almost as bright green as when first laid upon the soil.

Returning to Adelaide, it was surprising to find, on August 31, that every leaf of the series showed a distinct protuberance at or near the proximal end (Text-figs. 16–22). Three of the leaves were still lying horizontally; two had the proximal end slightly elevated; the three others were curved crescent-wise with both ends elevated.

The development after this date was comparatively rapid. On September 16 four of the leaves were removed, sketched under the dissecting microscope, and carefully replaced in their original positions (Text-figs. 16 A, 17 A, 18 A, and 19 A). They all showed irregular, greenish-yellow cell


17B
19B
20B
17A
19A
20A
18D
16B
18C
21
16A
18A
18B
22

masses. In one case (Text-fig. 16 A) this was all the development visible, but two of the others (Text-figs. 17 A and 18 A) showed in addition a minute white conical projection, arising from the cell mass and growing vertically into the soil. In one case (Text-fig. 18 A) rhizoids were visible arising from the cell mass close to the leaf. The fourth leaf (Text-fig. 19 A) showed a comparatively small cell mass, but from it there descended a cylindrical process, 2 to 3 mm. long, that terminated below the soil in a blunt apex. The structure resembled the stalk from which the new tuber of a *Phylloglossum* plant arises.

By September 24 the leaf shown in Text-fig. 17 A had made further growth, the growing-point observed upon it having given rise to a descending shaft. It was sketched (Text-fig. 17 B) and fixed for microtoming. At the same time it was noticed that the leaf shown in Text-fig. 18 A showed two new features. First, the growing-point, already observed upon it, was distinctly bilobed ; second, from the upper surface of the cell mass, a minute green leaf-like process was emerging (Text-fig. 18 B). The further history of this leaf is shown in Text-figs. 18 C and D, drawn October 8 and November 16 respectively. The green process developed into a slender 'leaflet' 4–5 mm. long, whilst each growing apex developed a white tuberous body at the end of a short stalk.

On October 8 two other leaves were found to have developed leafy processes, one of which is shown in Text-fig. 20 A. The figure also serves to indicate the extent of tuber formation by this date. There is a distinct stalk which carries the growing-point into the soil. The growing-point is swollen and whitish, but as yet quite smooth. The cell mass and such portions of the stalk as are exposed to the light are green. The presence of rhizoids upon the cell mass was found to be inconstant at this stage. Sometimes they could be distinguished, but more often they were absent. This, however, may have been due to injury, though every care was exercised in removing leaves for observation. Later, as will be seen, the tuber develops many rhizoids.

On October 19 one leaf of the series, that had previously been observed to be changing to a dull watery green, was quite collapsed. It

DESCRIPTION OF FIGS. ON OPPOSITE PAGE.

TEXT-FIGS. 16–22. Leaves of Series B, detached and put on soil July 11, 1918. The dates below in brackets are those on which the figures were drawn. All $\times 5\frac{1}{4}$. Fig. 16 A. Cell mass forming (Sept. 16). Fig. 16 B. Same with adventitious tuber (Nov. 16). Fig. 17 A. Growing-point just visible on cell mass (Sept. 16). Fig. 17 B. Same, showing elongation of stalk (Sept. 24). Fig. 18 A. Cell mass with rhizoids and short stalk bearing growing-point (Sept. 16). Fig. 18 B. Same developing two growing-points from cell mass, also 'leaflet' (Sept. 26). Fig. 18 C. 'Leaflet' enlarged, two tubers forming (Oct. 8). Fig. 18 D. Same; tubers are smaller and less regular than those formed singly by other leaves of the same series. Rhizoids absent (Nov. 16). Fig. 19 A. Small cell mass, stalk, and growing-point (Sept. 16). Fig. 19 B. Same (Nov. 16). Fig. 20 A. Portion of leaf showing cell mass. Leaflet stalk and tubers beginning to swell. No rhizoids at this date (Oct. 8). Fig. 20 B. Same original leaf beginning to rot (Nov. 16). Fig. 21 and 22. Two remaining leaves of series (Nov. 16).

was too rotten to remove for sketching, but, on examination, a white rounded body, barely 2 mm. in diameter, was found on the under side near the proximal end of the leaf. This was the only failure in the series, and it was not a completely negative result.

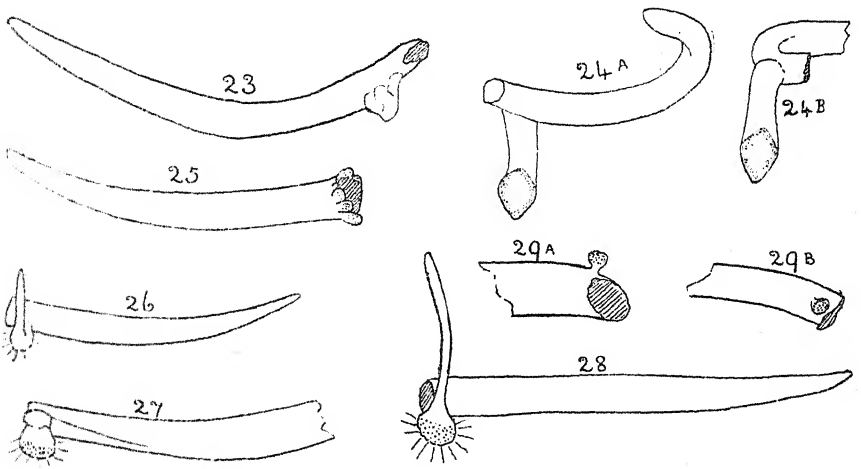
By November 16 it was found necessary to stop the experiment, and the remaining six leaves were all sketched before fixing. At this date the original leaves were yellowed, and though they still retained their shape they were found to be mere hollow shells, the mesophyll being quite collapsed. The leaflets also were yellowing, but the cell mass appeared turgid and green. Text-fig. 18 D shows that the two tubers developed from the single leaf were without any rhizoids. These tubers were opaque and white, but smaller and less regular than those formed singly by the other leaves. The remaining five leaves (Text-figs. 16 D, 19 B, 20 B, 21, and 22) all showed tubers, borne at the end of more or less elongate stalks, externally resembling the tubers normally produced by *Phylloglossum* plants, and having the usual abundant development of rhizoids that characterizes the later stages of tuber growth. In most cases (Text-figs. 16 B, 20 B, 21, and 22) the tuber was sunken for less than 4 mm. below the surface of the soil. In Text-fig. 19 B the stalk is seen to be much longer. As will have been noticed from Text-fig. 19 A, this leaf showed quite early in the experiment a pronounced upturning of the ends, so that it rested upon the soil by a small portion of its surface only. The resistance offered by the soil to the downward growth of the stalk caused the proximal end to be still further elevated, thus the stalk became unusually elongated.

(ii) *Series C.*

On July 21 ten leaves were broken off plants of *Phylloglossum* growing in a sod of soil as before, and laid horizontally upon it. In this experiment the sod was placed in a large china developing dish and covered with a piece of plate-glass, in order to secure better illumination. Under these conditions the atmosphere was at a less constant humidity than in Series B, for the glass plate did not fit tightly. While care was taken to prevent the soil drying up, no precautions were taken to keep it constantly damp, for it was felt that by allowing drier periods to alternate with wet ones, field conditions would be more closely reproduced.

It is unnecessary to describe the results in detail. The leaves, as in Series B, showed no visible change other than curving until after August 15. By August 31 several were noticed to be forming cell masses near the proximal end, and one was removed for microtoming. It then showed an irregular nodular swelling, yellowish in colour (Text-fig. 23). Three other leaves were removed early in October. Two of these showed irregular cell masses, from the bases of which white growing apices were projecting.

These subsequently proved to be the growing-point in process of invagination.[1] The third leaf showed signs of damping off, but it had a short stalk bearing a slightly swollen tuber, enclosed in which the growing-point could be discerned. No rhizoids were present at this stage. All the others appeared healthy, but showed, with one exception, little development above ground. The exception had two 'leaflets' about 4 mm. long, one arising on each side of the leaf and springing from the lower side. Unfortunately, when examined three or four days later, this leaf had collapsed too much to draw. Each leaflet appeared to be attached directly to the main leaf, with no noticeable swelling on or beneath the surface of the soil.



TEXT-FIGS. 23–29. Leaves of various series. The dates below in brackets are those on which the various figures were drawn. All × 5¼. Fig. 23. Leaf of Ser. C. The cell mass is nodulose; a T.S. of this leaf is seen Fig. 38 (Sept. 24). Fig. 24 A and B. Leaf of Ser. C. This leaf curved on soil in horizontal plane. Adventitious growth from convex side (Nov. 16). Fig. 25. Leaf of Ser. D. Note absence of definite growing-point, and formation of many starchy cell masses (Oct. 21). Figs. 26–29. Leaves of Ser. E; for further explanation see text (Nov. 25).

By November 16, almost four months after the experiment was started, the remaining leaves were seen to be yellowing rapidly. They seemed to be mere hollow shells for the greater part of their length, and collapsed when lifted. The base of the leaf, however, was still green, as was the small cell mass. The leaves were all curved, four with their apices erect; the fifth lay curved C-shaped on the soil. In this case also the adventitious growth was from the convex side and so placed that the cell mass originally would not have been in direct contact with the soil (Text-fig. 24 A and B). Each of the leaves had developed a short stalk bearing a tuber, on which rhizoids were just appearing.

[1] See p. 504 below.

(iii) *Series D.*

This series was grown on a similar sod of soil to Series C and in the same dish. It was, however, much less successful. The leaves were often buried by the castings of small animals or overgrown by Bryophytes. Two leaves fixed early in October presented a similar appearance to that shown in Text-fig. 23. By the end of the month six of the remainder had damped off. Only one of these showed any adventitious growth, a whitish swelling about 1 mm. in diameter near the proximal end on the lower side. The last two leaves were fixed at the end of November, when one showed a dropper, the apex of which was hardly swollen. The other had a series of nodular swellings extending almost around the leaf near the cut surface; several of these appeared white, like minute tubers (Text-fig. 25).

It is difficult to account for the comparative failure of this series, unless it were due to competition with various organisms.

(iv) *Series E.*

This series was not started until September 13, when twelve leaves were laid on soil in a glass dish as above. Three of these collapsed in about a fortnight, and five others before the end of November. Of the remaining four, three (Text-figs. 26–28) each formed a small irregular cell mass near the distal end. Two of these (Text-figs. 26 and 28) produced 'leaflets', and all showed rhizoids, but no dropper was developed. The cell masses themselves appeared opaque white, like resting tubers. The fourth leaf (Text-fig. 29) only produced a minute white structure about 1 mm. diameter. This swelling was developed from the surface of the leaf remote from the soil.

(v) *Series A.*

The consideration of this series has been postponed until the other experiments had been described, because, of the eight leaves used, only one formed a single tuber (Text-fig. 30) in the manner described above. All the remainder produced abnormalities.

As has been explained, the conditions of the experiment were slightly different, in that soil from the locality in which *Phylloglossum* was growing was placed in a Petri dish and lightly tamped down, instead of using an undisturbed sod. Water was sprayed over the soil from time to time to keep it moist. Though the experiment was begun on June 13, nearly a month before any other series, no development occurred until the latter end of August, by which date all other series also showed growth. The only noticeable feature displayed by the leaves during the first two months was the remarkable curving exhibited by them. With a single exception, in which case the leaf lay flat along the soil throughout the five months the

12. DEC

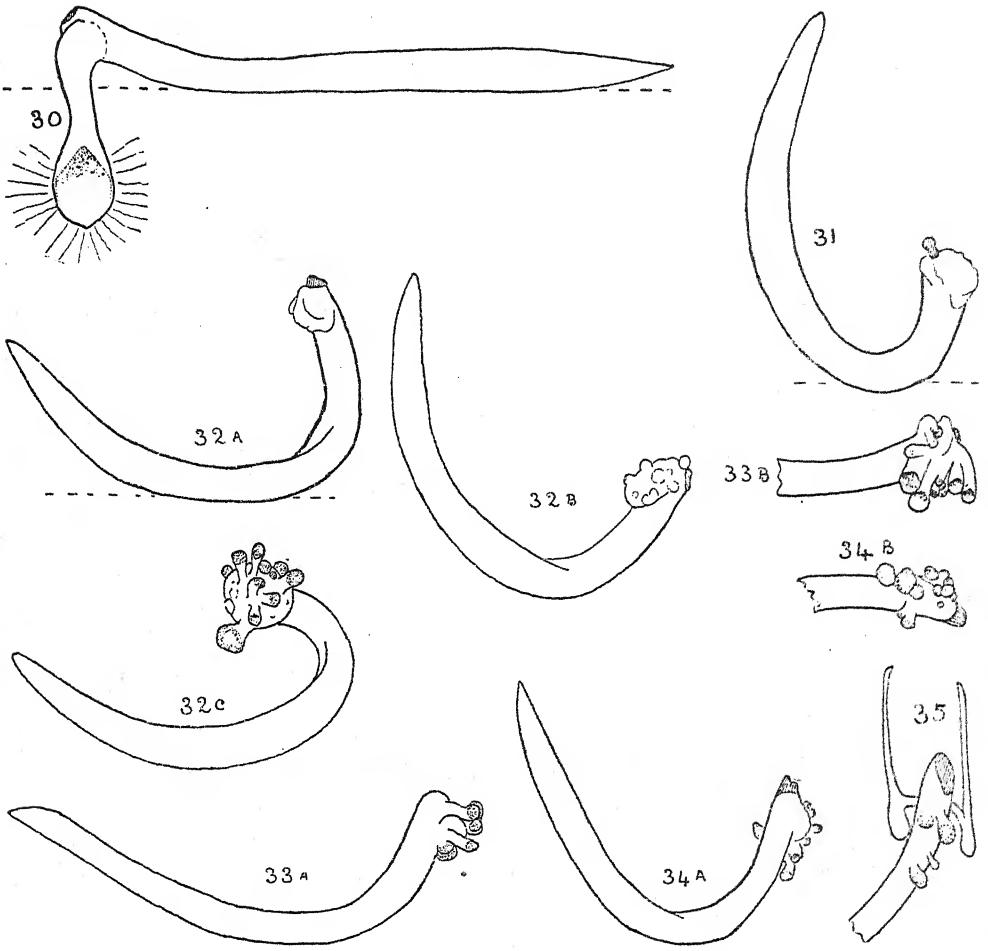
experiment lasted, all the leaves early began to curl upwards, becoming J- or U-shaped as one or both ends were elevated.

The curling was not due to the effect of light, for frequently the proximal end was elevated, whilst the apex showed no response. It was noticed that in dry weather the leaves of plants growing in the field might show a marked incurving of their tips. It therefore appeared probable that the curving was due to some alteration in the turgidity of the leaf. This point is further discussed later (p. 510).

By August 31 all the leaves showed more or less adventitious growth near the proximal end. Most of the cell masses were lifted above the soil owing to the curvature of the leaf. All of them had an irregular contour. The appearance about this date is shown in Text-figs. 31 and 32 A, drawn September 16. As it was feared that the cell masses might dry up because of the elevation of the proximal ends, some of the leaves were laid flat upon the soil. On October 21 the leaf shown in Text-fig. 32 A was sketched again (Text-fig. 32 B). It will be seen that the cell mass now showed distinct scattered nodules. These were green, no white growing-point being seen.

By the middle of November it was necessary to stop the experiment. Only one leaf (Text-fig. 30), that which lay horizontally, showed a single tuber resembling those formed in the other series. Text-fig. 32 C shows the condition of the leaf drawn in Text-figs. 32 A and B. The nodular growths noticed before had most of them elongated to form minute stalks terminating in slightly swollen white heads. Text-fig. 33 A shows another leaf viewed from above. The cell mass is seen to be near the proximal end, but on the convex side of the curved leaf. Stalklets bearing small white heads are visible. Viewed from the side (Text-fig. 33 B) it is seen that these minute tuber structures, developed irregularly over the cell mass, are markedly geotropic in their growth, though several of the stalks were not sufficiently elongated to bury their apices. The leaf shown in Text-fig. 34 A (also drawn from above) had its apex erect. From the under surface of this (Text-fig. 34 B) many irregular white nodular structures of various sizes were developed.

The leaf shown in Text-fig. 35 is interesting. In this case the adventitious cell masses were scattered over a greater length of leaf than is usual. The majority of them merely developed short stalks in the white swollen heads. Two of the growths, however, developed leaflets as well as descending portions. By November 17 the original leaf was obviously dying off, so that it was necessary to fix it, and further development could not be followed. It will be noticed, however, that each leaflet-bearing structure resembles that shown in Text-fig. 20 A, but at an earlier stage.



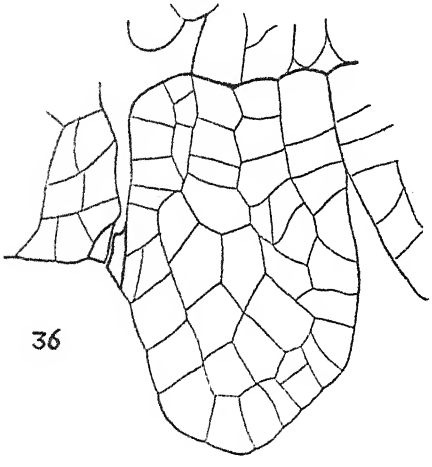
TEXT-FIGS. 30–35. Leaves of Series A, detached and put on soil, June 13, 1918. The dates below in brackets are those on which the figures were drawn. All × 5¼. Fig. 30. Adventitious tuber arising from side (Nov. 16). Fig. 31. Extreme case of leaf curvature in vertical plane. Cell mass nodular (Sept. 16). Fig. 32 A. Leaf showing vertical curvature. This leaf appears to be an exception to rule that adventitious growth occurs from convex surface (Sept. 16). Fig. 32 B. Same, viewed from above; note nodular development of cell mass. This leaf was laid flat on soil Sept. 16 (Oct. 21). Fig. 32 C. Same; note many short-stalked, white, swollen bodies (Nov. 27). Fig. 33 A. Leaf viewed from above, and B, viewed from side, showing geotropic curvature of the many minute, stalked tubers (Nov. 27). Fig. 34 A. Leaf viewed from above, and B, viewed from below (Nov. 26). Fig. 35. Portion of leaf, viewed from side, with several scattered centres of adventitious growth. Two have developed leaflets and small tubers (Nov. 17).

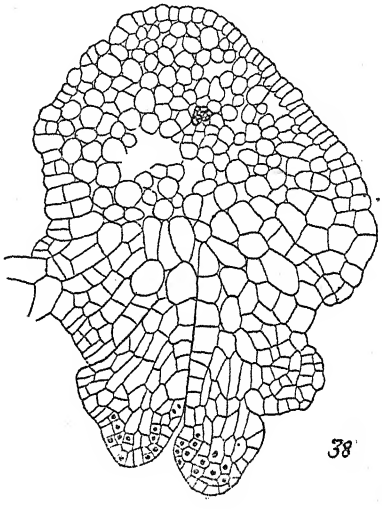
Developmental History.

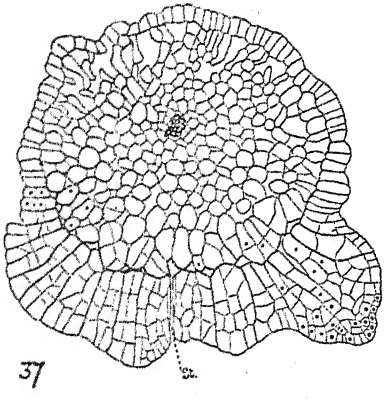
The first stage in adventitious tuber formation is the development of a pad of tissue or cell mass, projecting from the epidermis, at or near the proximal end of the detached leaf. This projection is due to the activity of certain epidermal cells; usually several are involved, but it would appear

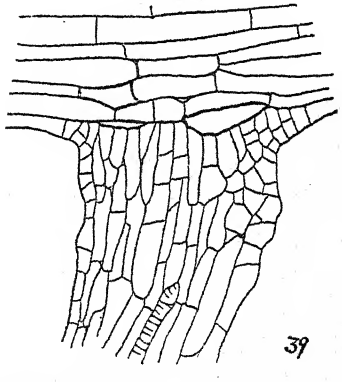
from those abnormal cases in which a number of minute tuberous bodies is formed (e.g. Series A) that each may arise from the activity of a single epidermal cell (Text-fig. 36).

The epidermal cells, which are considerably elongated in the direction









TEXT-FIG. 36. Portion of transverse section of leaf showing adventitious growth from single epidermal cell. Stomate seen to left. × 180.

TEXT-FIG. 37. Transverse section of leaf showing considerable cell mass of epidermal origin, interrupted by stomate at *St.* Growth is becoming localized by development of growing-point seen at right. × 44.

TEXT-FIG. 38. Transverse section of leaf (see Fig. 23). Two growing-points are developing; the anticlinal walls separating the groups of epidermal cells involved are plainly seen. × 44.

TEXT-FIG. 39. Portion of longitudinal section showing junction of leaf tissue and cell mass showing epidermal origin of latter. Cell mass developed stalk in which a 'tracheide' is seen. × 44.

of the leaf axis, first divide transversely, and at the same time the segmented cells enlarge in a radial direction. The radial elongation is accompanied by periclinal division.

The evenness of contour of the cell mass thus produced depends on how nearly equal the stimulus to division has been in neighbouring cells, and upon the absence of stomates from the proliferating area (Text-fig. 37 and Pl. XXVIII, Photo 1). In Text-fig. 38 and Photo 2 it is obvious that growth has been less regular, the boundary between original groups of epidermal cells being clearly shown by anticlinal planes of division, extending for as many as eight or nine cell rows. There is thus developed a considerable cell mass due entirely to proliferation of the epidermis. Concurrently the sub-epidermal cells enlarge somewhat, and a few irregular walls are formed. These are best seen in a section tangential to the leaf-surface, when they resemble the secondary changes that may occur in the cortex of some herbaceous plants (Photo 4). In no case has the sub-epidermal tissue been observed to take any part in the structure of the main adventitious cell mass (cf. Text-fig. 39 and Photo 5), though in cases where the growth is close to the injured surface the activity of the sub-epidermal cells is greater than usual (Photo 4). The changes that occur within it are of such a nature as to reduce the amount of intercellular space below the adventitious mass, thus permitting of freer translocation between it and the leaf.

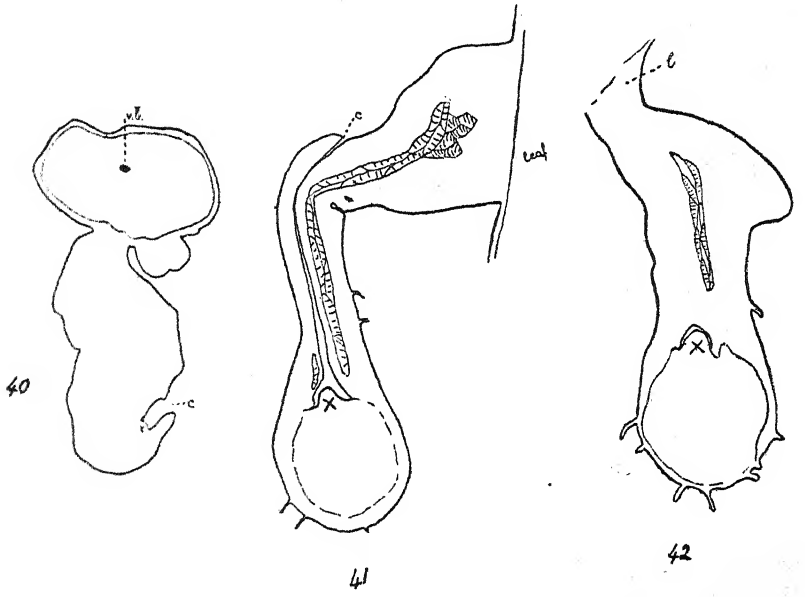
The size of the cell mass produced appears to be directly related to conditions of growth. In contact with the soil or in very humid atmosphere, it may be small in amount; in drier conditions growth is slower and a considerable and very irregular mass may be produced before further differentiation proceeds. A further factor, that of the age of the regenerating leaf, is discussed below. It has already been noticed that rhizoids were not of constant occurrence upon the cell mass, though they invariably occur in large numbers upon the tuber in the later stage of development.

Once the cell mass is formed the next stage is the localization of growth at one or more centres by the differentiation of growing-points (Text-figs. 37–8 and Photo 3) consisting of a group of meristem cells. The growing-point is definitely geotropic, and quickly becomes buried in the soil. When, owing to its growth, the apical meristem has become buried, an invagination of the growing-point occurs as a result of unequal growth. This is well shown in Text-fig. 40 and Photo 6, which shows the growing apex already invaginated, and lying at the base of a short pit. In the case of this leaf a considerable irregular cell mass was formed owing to there being more than one centre of proliferation. However, only one growing-point developed.

The subsequent growth of the stalk, after invagination of the apex, resembles that described for the normal new tuber. The apex is buried by intercalary growth of the stalk cells, which become considerably elongated in the vertical plane (Text-fig. 39 and Photos 5, 8, and 9). As in the normal tuber, the apex is in communication with the exterior along a channel ('Canal de Braun' of Bertrand) (Text-fig. 41). This, as usual,

dilates at the distal end to form a chamber into which the growing-point projects (Text-figs. 41 and 42 and Photos 7, 8, 9, and 11).

In the majority of cases it is not until the apex is well buried that growth takes place in all directions, resulting in a tuber. This has the usual conical meristem seated on a spherical mass of parenchyma (Photo 7). All the essential features of a normal tuber of *Phylloglossum* are shown in this section made from a detached leaf after three months' growth. At this



TEXT-FIG. 40. Transverse section of leaf; epidermis indicated by faint line. An irregular cell mass has formed, and the growing-point has begun to invaginate. *c.* = opening of channel, *v.b.* = vascular supply of leaf. Camera lucida outline. × 26.

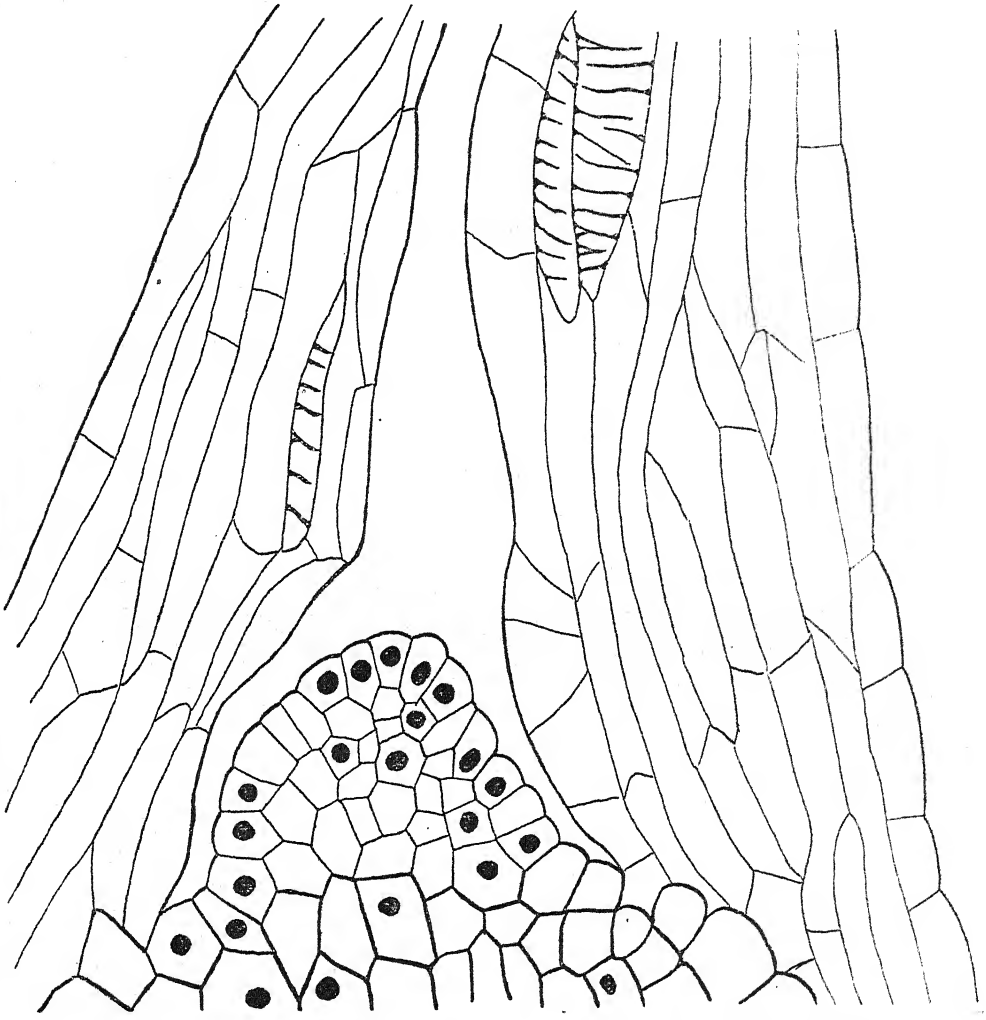
TEXT-FIG. 41. Portion of longitudinal section through cell mass, stalk (which was accidentally bent), and tuber. 'Vascular supply' seen from its expansion in cell mass to cup of 'tracheides' around growing apex (*x*). Apex projecting into the chamber and seated on spherical mass of starchy tissue. The whole length of channel shown. *c* = opening of channel. Camera lucida outline. × 26.

TEXT-FIG. 42. Longitudinal section of adventitious growth on leaf in Fig. 20 B. Apex (*x*) seen projecting into chamber; extent of starchy tissue shown by faint line. Greater part of 'vascular supply' seen, extending towards 'leaflet' (*l.*) (which passes out of plane of section), but no 'leaflet' bundle differentiated. Camera lucida outline. × 26.

stage, however, tuber and dropper consist entirely of parenchyma. Subsequently a 'vascular supply' is developed and differentiation proceeds within the tuber.

The 'vascular supply' consists of a strand of tracheidal cells, with loose spiral or reticulate markings, extending from the original cell mass along the dropper to a point at the level of the apical meristem. This development is most active during the last month of growth, and results in the lignification of certain of the parenchyma cells. These agree with their

neighbours in size and shape, hence in the cell mass the 'tracheides' are more or less isodiametric (Photo 10), while in the dropper they are elongate. Within the adventitious cell mass the 'tracheides' form a broad core



TEXT-FIG. 43. Detail of apex of tuber seen Fig. 41. Starchy tissue indicated by heavier outline to cells. × 270.

extending towards the original leaf but not reaching so far as sub-epidermal tissue, hence, of course, they never form a connexion with the leaf trace. In the tuber stalk it is interesting to observe the vascular supply may expand to form a cup of 'tracheides' around the apex (Text-fig. 43), as has been described in the normal tuber.[1] Whilst lignification is

[1] Wernham, H. F. (1910) : loc. cit., p. 388.

proceeding the tuber becomes further differentiated. The outer layer or coat consists of rather large cells with stout cell walls, and numerous rhizoids are developed (Photos 8, 9, 10, and 11). The coat rarely is more than one cell thick, and in irregular tubers may be absent. In this respect it differs from the new tuber coat described by Bower,[1] which consists of two or three cell layers besides the epidermis, the latter having characteristically thickened walls and rhizoid-like hairs. The tissue comprising the main body of the tuber consists entirely of parenchyma, with few intercellular spaces. This tissue is packed with minute starch grains, and forms an almost spherical body of cells, near the upper pole of which the growing apex is found. The cells of the apex are clearly differentiated from the rest of the tuber by their large nuclei, abundant protoplasm, and the absence of cell inclusions. The appearance of a fully-developed adventitious tuber is well shown in Text-figs. 41, 42, and Photos 8, 9, 11, especially Text-fig. 41, which shows the 'tracheide' strand from its expansion in the adventitious cell mass, throughout its whole length, to the cup around the tuber apex. The same section also shows the channel throughout its whole length, including the rather prominent opening to the exterior (see also Photo 10). The position of this shows that invagination of the apex did not occur till after an appreciable amount of geotropic growth had occurred. Only two adventitious tubers showing 'leaflets' developed from the cell mass have been examined in serial section. The 'leaflet' structure is exceedingly delicate, and consists of an epidermis, a spongy mesophyll, with large intercellular spaces and a central strand. In one 'leaflet' this central strand consists of elongate parenchyma only, and, as the main leaf had already begun to rot when fixed, little further development could have occurred. In the other case there was a well-developed vascular strand seen in transverse section to consist of 4–6 larger 'tracheides' around two or three smaller ones. This vascular strand passed down the leaflet and connected with the tracheidal cells in the cell mass. It was noted that certain epidermal cells of the leaflet near to its apex developed fine rhizoid-like processes.

General Results and Conclusions.

The observations on the behaviour of the tuber of *Phylloglossum* recorded in the opening sections of this paper serve to emphasize the importance of the structure as an organ of perennation. This view, referred to by Professor Bower in his Presidential Address to Section K of the British Association, 1914,[2] expresses more nearly the true value of the tuber than one which regards it as an annually produced protocorm developed by a permanently embryonic Lycopod.

[1] Bower, F. O. (1885): loc. cit., p. 666.
[2] Bower, F. O. (1914): Brit. Ass. Report, p. 565.

In South Australia the geophytic element in the flora of certain areas is very large.[1] Physiologically the tuber of *Phylloglossum* is comparable with the tubers, corms, &c., of the spermophytes among which it grows. Like them, it has an average depth to which the perennating organ is sunken, a depth that it maintains in spite of accidental circumstances that tend to bury or expose the tuber. In this respect its behaviour agrees with that of the geophytes of other countries.[2]

The analogy drawn by Wernham[3] between the tuber of *Phylloglossum* and the stalked droppers of *Tulipa* and *Erythronium* described by Mrs. Arber[4] seems particularly apt, though other workers have found nothing to justify his conclusions as to the morphological nature of the tuber.

It may naturally be asked how far the development of adventitious tubers upon leaves is a normal occurrence and so of value for purposes of vegetative reproduction. Unfortunately no definite answer could be given to such a question. In 1917 seven leaves in various stages of tuber formation were collected in the field. Had these been left undisturbed, possibly not more than two of them, those shown in Text-figs. 8 and 12, would have developed sufficiently to be of value for vegetative propagation before the oncoming dry season put a stop to all growth. On the other hand, in certain seasons the district near to Adelaide in which *Phylloglossum* grows may remain green and growth be possible until the end of December. In

RAINFALL IN INCHES AT BELAIR, SOUTH AUSTRALIA.
ALTITUDE 1,009 FT.

Year.	*Jan.*	*Feb.*	*Mar.*	*Apl.*	*May.*	*June.*	*July.*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*	*Total.*
1915	0·62	0·03	0·46	2·58	4·20	6·92	3·88	4·67	5·09	0·94	0·36	0·07	29·82
1916	0·65	0·12	0·40	2·18	1·20	11·08	3·55	5·69	1·96	2·41	4·08	1·26	34·58
1917	0·50	2·77	3·27	1·39	6·92	4·53	5·91	4·41	5·54	3·10	1·23	1·29	40·86
1918	0·63	0·68	0·46	1·58	3·86	4·88	3·32	4·14	1·35	4·39	0·45	0·60	26·34
Average Rainfall for 38 years	0·99	0·65	1·39	2·59	3·39	4·95	3·55	3·52	2·81	2·24	1·43	1·12	28·63

such circumstances there is a reasonable chance that most leaves which began to form tubers would mature them. In this connexion it is interesting to recall that in some spots a considerable percentage of single-leaved plants was observed at the close of the 1917 growing season.[5] Many of these plants were very minute, yet all were from tubers of a preceding season. The year before, 1916, was unusually wet during November.[6] It

[1] Osborn, T. G. B.: loc. cit., p. 9.
[2] Goebel, K. (1905): p. 466.
[3] Wernham, H. F.: loc. cit., p. 343.
[4] Robertson, A. (1906): Ann. Bot., xx, p. 429.
[5] Osborn, T. G. B.: loc. cit., p. 4. Of 184 plants 39 had one leaf only.
[6] I am indebted to Mr. E. Bromley, State Meteorologist, Commonwealth Meteorological Bureau, who has kindly furnished the rainfall records at Belair which are given above.

is possible that some of these single-leaved plants were formed from leaves detached during the 1916 growing season, especially as in many instances the tubers from which they arose were very small and placed near to the surface of the soil. When plants of *Phylloglossum* are turgid their leaves are brittle and easily broken through. Apart from animal agency, a blow from a falling twig or even a wind-swept leaf of *Eucalyptus* might detach one.

The production of adventitious tubers by leaves of *Phylloglossum* has an interest quite apart from considerations of the morphology of the plant producing them, namely, as an instance of regeneration. It will have been observed that the adventitious cell mass produced on injured leaves invariably arises at or near to the proximal end of the leaf. In this respect it differs from the position of the cell mass formed in regeneration from detached leaves of *Lycopodium ramulosum* described by Holloway.[1]

There the leaf shows no polarity and the adventitious structure may be produced from any point. However, this may result from the small size and apparently unspecialized nature of the leaf in question. In *Phylloglossum*, the consistent development of the adventitious growth at the leaf base agrees with Goebel's generalization—'that the place for the formation of new organs in regeneration is definite and is primarily dependent upon the direction in which the plastic substance moves in the uninjured plant.'[2]

But a further consideration arises which is obvious from a study of the figures, namely, that while the cell mass invariably arises near the leaf base, it is apparently immaterial upon which side it develops in relation to the soil. In most cases it is developed from the side of the leaf next the soil, even when this is elevated by curvatures of the leaf well above the wet ground. But Text-figs. 12, 24, 33, show that it may be lateral to the leaf as it lies on the soil, and in one case, Text-fig. 29, it is from the upper side, i.e. that remote from the soil. Moreover, it has been noted that, with a single possible exception, the adventitious growth in those leaves which exhibit curvature is always from the convex side. Hence it seems that it is not merely the stimulus of contact with the damp ground which determines the position of adventitious growth, but a morphological factor must be taken into consideration as well. Unfortunately it was not thought necessary to mark the morphological upper surfaces when detaching the leaves, and, as these appear nearly cylindrical, it is difficult to recognize afterwards, but it is possible to arrive at some conclusion from a consideration of structure. The anatomy also serves to explain the curving of the leaves to which reference has already been made.

The main features in the structure of the leaf are already well known.

[1] Holloway, J. E. (1916): Trans. N.Z. Inst., xlix, p. 88, Figs. 14–20.

[2] Goebel, K. (1900): p. 45.

In section it is said to have an almost circular outline.[1] This is true of the leaf for the greater part of its length, but towards the base it is usually decidedly elliptical and sometimes becomes, by compression against the other leaves, segment-shaped. The usual result of this is that the leaf, when detached, lies either on the abaxial or adaxial surface, generally the latter, aud seldom on its side. Further, the simple leaf bundle, which is said to lie in 'the centre of the transverse section', only has this position in the upper portion of the leaf. Towards the base it lies on the minor axis of the ellipse but displaced from the median line, being nearer to the adaxial surface (Text-fig. 40 and Photos 2 and 6). More than 'four or five xylem elements' have been found in transverse section; the number is usually ten or more near the base. This eccentric position of the leaf bundle within the somewhat flattened leaf has the effect of producing curvature when turgidity is reduced, and it is the abaxial surface which becomes convex under such circumstances. The inference, therefore, is that the adventitious growth occurs from the abaxial surface. This conclusion is supported by the transverse sections seen in Text-figs. 37, 38, and 40 and Photos 2 and 6. It is less easy to judge from longitudinal sections, in which plane most of the leaves were cut, but Photo 7 supports this conclusion.

The amount of adventitious growth made by the leaves of the various series was markedly different. The difference can be correlated with the period of the growing season at which the leaves were detached. The series may be conveniently placed in three groups: A, started early in the growing season (June 13), a little after the tips of the largest leaves were noticed above the soil; B, C, and D, started in July, when the plants had been growing for at least six weeks in the field; and E, started two months later (September 13). Series B and C were very successful.[2] Of the 18 leaves involved, 12 matured tubers and 3 others would probably have done so had they not been removed for sectioning before the tubers were fully developed. The remainder all showed some growth. Series E, on the other hand, was a comparative failure. Unlike the leaves of the other series, which remained green for four months or longer, many of the leaves of this series quickly rotted away. Four only formed any growth, and in the case of one of these it was very slight. The remaining three leaves only developed as far as the cell mass; this, however, became white and starchy and in two instances formed a 'leaflet'. During the two months, July 21 to September 13, which elapsed between starting Series C and E, considerable development occurred in the new tubers formed by plants growing in the field. In July the new tuber merely showed a stalk in process of elongation with a small unswollen apex. By September the stalk had reached its full

[1] Bower, F. O. (1885): loc. cit., p. 673. The other quotations in this paragraph are from the same source.

[2] It seems legitimate to neglect Series D, since its failure may be attributed to other reasons than the age of the leaves.

length and the new tuber was appreciably swollen, showing that considerable plastic material had been transferred to it. By the latter date the leaves of the growing plant were presumably depleted, although they did not appear senile, and the phase at which regeneration could be successfully accomplished was nearly over.

In regeneration a cell mass is invariably formed first. Upon this a growing apex usually differentiates, from which the starch-containing tuber develops. But in some cases no apex is formed and the cell mass itself becomes starchy as the growing season concludes (Series A and E). Thus the cell mass may, under some circumstances, behave as a perennating organ. The essential feature of the cell mass is that it is a roughly spherical structure, in which carbohydrate material may accumulate, but which is capable of producing further growth after differentiating a growing-point. The occurrence of a 'leaflet' upon the cell mass is variable. In Series E two of three leaves developed one, but in Series B the three leaves that did so produced the 'leaflet' only after a considerable amount of adventitious growth had taken place. This suggests that the 'leaflet' is a structure of secondary importance, produced in response to some such physiological stimulus as a failing or insufficient carbohydrate supply. The cell mass is, as it were, the first foothold secured by a young plant, a starting-point from which further developments may occur, circumstances being favourable. Small parenchymatous swellings containing starch are not uncommon amongst minute plants in regions of rapidly varying humidity. A species of *Fossombronia* growing beside *Phylloglossum* has a thallus with a markedly swollen midrib. The tubers of certain Australian species of *Anthoceros* are well known. Near to the locality in which *Phylloglossum* occurs, *Anogramme leptophylla* grows in abundance. This has a peculiar prothallus with a small, starchy tuber.[1] Bearing in mind the climatic conditions under which it grows, the early and constant production of a cell mass gives special interest to the behaviour of regenerating leaves of *Phylloglossum*, and to any comparison that may be made between them and those of certain species of *Lycopodium*.

Cases of vegetative reproduction in the genus *Lycopodium* are well known, the example of adventitious shoots produced by the first leaves of embryo plants of *Lycopodium inundatum* cited by Goebel[2] being of special interest in considering the behaviour of *Phylloglossum*. Our knowledge of vegetative reproduction and regeneration within the former genus has been considerably extended of late by Holloway.[3] Specially valuable for comparison with the structures described in *Phylloglossum* are the gemmae produced from old roots and from detached leaves of *L. ramulosum*. The gemmae[4] formed from detached fragments of roots are stated to arise from

[1] Goebel, K. (1905): p. 216.
[2] Goebel, K. (1900): p. 46.
[3] Holloway, J. E. (1916): loc. cit.
[4] Loc. cit., p. 85.

cortical cells. The youngest stages show the development of transverse walls in certain cells from which a cell mass subsequently forms, which swells externally and produces a 'protophyll'. The following account [1] of development of leaf bulbils is given. 'An adventitious bud on a leaf shows first as a small, roundish, green cushion of meristematic tissue which has originated probably from one or more epidermal cells. This cushion develops into a roundish or egg-shaped cell mass which gradually elongates, and on which at an early stage rhizoids arise. The attachment of the young bud . . . shows that the main tissue of the leaf is undisturbed.' These buds produce one to three 'protophylls' and are firmly attached to the substratum by rhizoids. Both 'protophyll' and swelling are packed with starch grains, especially the latter, which develops a storage tissue. The parent leaf is 'greenish at its upper portion but yellowish below and is always obviously broken off at its lower extremity'. The bulbil is subsequently freed by rotting away of the leaf. These bulbils Holloway compares with the protocorm of *L. cernuum.*

If the term 'protocorm' is to have any morphological value it is well that it should be limited to certain structures such as the extra-prothallial swelling of the embryo of *L. cernuum* and to the similar structures formed in *L. laterale* [2] and *L. ramulosum.*[3] In the last two species the protocorm is of unusual size, but Holloway points out that in this large structure two portions may be recognized, 'the original protocormous tuber surmounted by its two protophylls' and 'the rhizomatous extension—an added feature to be interpreted apart from the original tuber'. From an examination of material of *L. laterale*, collected by him in Tasmania, the writer is inclined to agree with this distinction. It is thus 'the protocormous tuber' of *L. laterale* which is the homologue of the protocorm of *L. cernuum.* However, seeing 'that vegetatively produced plants (from bulbils, gemmae, &c.) tend in their development to pass through stages in elaboration similar to young plants developing from spore or zygote',[4] Holloway's extension of the term protocorm to the starch-containing bulbils of *L. ramulosum* and *L. laterale* seems perfectly justifiable. But it is unlikely that the protocorm, even so defined, has any phylogenetic significance.[5] It seems preferable to regard it as 'an opportunist local swelling . . ., which, though biologically important . . . is not really primitive.' [6]

The biological importance of the protocorm becomes increasingly obvious as our knowledge of the ecology of those species of *Lycopodium* which produce it is enlarged. From Holloway we learn that the three New

[1] Holloway, J. E. (1916): loc. cit., p. 88.
[2] Holloway, J. E. (1914): Trans. N.Z. Inst., xlvii, p. 73, and ibid. (1915), p. 277.
[3] Holloway, J. E. (1915): loc. cit., p. 285.
[4] Lang, W. H.: Brit. Ass. Report, 1915, p. 706.
[5] Bower, F. O. (1908): p. 225.
[6] Bower, F. O. (1914): p. 565.

Zealand species of *Lycopodium* which form protocorms all have short-lived prothalli[1] and grow in localities that are subject to summer drought.[2]

In *Phylloglossum* the prothallus is stated to be of the *L. cernuum* type,[3] whilst the climatic conditions under which it grows are also those of a winter rainfall and a dry summer period. The gametophyte generation and the conditions of growth are thus similar to those of the protocorm-producing species of Lycopods. It is not surprising, then, that *Phylloglossum* should produce a protocorm, since this structure appears to be essentially of biological importance in the life-history of plants having a short-lived prothallus of the *L. cernuum* type and growing under special climatic conditions. Many workers have regarded the tuber of *Phylloglossum* as a protocorm, but this is hardly a legitimate use of the term. By comparison with species of *Lycopodium* it will be recognized that it is the cell mass formed by regenerating leaves, and not the tuber which may arise from it, that must be considered as the protocorm, since it corresponds to the protocorm of *Lycopodium* spp. in developmental history and in function. Unfortunately the embryology of *Phylloglossum* is as yet imperfectly known, but such evidence as we have indicates that a swollen cell mass is formed before the initiation of the tuber.[4] Miss Sampson has recently described a sporeling of *Phylloglossum*, and from her account [5] it is obvious that there is both 'an embryonic swelling' and a tuber. Unfortunately the account was limited to a single specimen, and it could not be determined whether the embryonic swelling was epibasal in origin, corresponding with the protocorm of *L. cernuum*. This seems probable, and there can be little doubt that biologically it is similar, functioning as a primary storage structure.

In the tuber of *Phylloglossum* we have a different structure. It has repeatedly been emphasized that, in the process of vegetative reproduction, tuber and cell mass or protocorm are two structures, the former arising from the latter only if conditions be favourable for continued growth. Such meagre facts as we have of the embryology are capable of a similar interpretation. The tuber is something extra, a structure in which *Phylloglossum* has 'bettered' the mode of life of certain species of Lycopods[6] (e.g. *L. inundatum*, which annually dies off to the tip of its shoot), since a definite tuber is unknown in that genus. Borrowing a term from another branch of biological science, the tuber may be regarded as a character of considerable 'survival value', for the climatic and edaphic conditions under which *Phylloglossum* has been studied are severe; but it seems hardly legitimate to style the tuber of *Phylloglossum* a 'protocorm' if any morphological value is to be retained for that term. That there is a distinction between these two organs is borne out to some extent by their differing

[1] Holloway, J. E. (1915): loc. cit., p. 276.
[2] Loc. cit., p. 263.
[3] Thomas, A. P. W.: loc. cit., p. 289.
[4] Thomas, A. P. W.: loc. cit., p. 288.
[5] Sampson, K.: loc. cit., p. 607.
[6] Bower, F. O. (1914): loc. cit., p. 565.

response to the stimulus of gravity. It has been pointed out that the new tuber is definitely geotropic. The adventitious tuber behaves similarly, but the cell mass or protocorm, which is developed first by the regenerating leaf, does not. It may grow from any side of the leaf in relation to the ground, and, even when developed in contact with it, shows no penetration into the soil beyond such amount as is due to expansion of the group of cells. The cell mass is a transitory structure and rarely attains any size, but where, as in Text-fig. 12, an unusually large one has formed, its growth is plagiotropic rather than geotropic.

It is not intended to discuss the morphology of the tuber at any length, but the following remarks may be deemed pertinent. In 1886 it was shown by Bower that the new tuber in sterile plants is formed directly from the apex of the plant, while in fertile plants, the apex of which develops the peduncle and cone, the new tuber is an adventitious growth bearing no relation to the leaf arrangement. This is substantially our position to-day, though recently Miss Sampson[1] has on anatomical grounds attempted to show that in fertile plants the apex bifurcates and that the new tuber is of the nature of a branch. The vascular anatomy of a minute plant such as *Phyiloglossum* is subject to considerable variation, as was shown by Bower.[2] The writer has hardly examined sufficient plants in serial section to be able to affirm or refute Miss Sampson's contention, but it seems significant that in two fertile plants recently studied no connexion existed between the vascular supply to the new tubers and the stele of the plant producing it. In one case, a four-leaved plant with two roots, the vascular strand from the new tuber completely died out in the stem before the xylem masses of the two roots coalesced, and far below the point to which any leaf trace penetrated. It is therefore hardly legitimate to assume that in all cases the tuber of fertile plants of *Phylloglossum* is a modified branch, or, indeed, that it represents 'a highly specialized leafy axis'. The 'vascular supply' to the adventitious tuber has been shown to be a late development and in no case connected with the tracheides in the regenerating leaf. The wide tracheidal cells of the 'vascular supply', with their loose spiral or reticulate lignification, resemble rather 'storage tracheides' than conducting elements. Such a function affords a reasonable explanation of the cup of tracheides formed at the distal end of the stalk round the new tuber apex. The reproduction of tubers by detached leaves agreeing, even to details of vascular supply, with those formed normally by the plant in each growing season, suggests that caution is necessary before adopting a generalization as to the morphology of the tuber which is based entirely on anatomical evidence.

[1] Sampson, K. (1916): Ann. Bot., xxx, p. 331.

[2] Bower, F. O. (1885): loc. cit., p. 674, and Pl. 70, Figs. 42, 43.

Summary.

1. *Phylloglossum Drummondii* occurs in South Australia as a member of the geophytic element in the flora of an area subject to prolonged summer desiccation.

2. The examination of a number of living plants has shown that, whilst the average depth of the current tuber is about 1 c.m., owing to various accidental causes it may range from a surface position to a depth of at least 2 cm.

3. Whatever the depth of the current tuber, the growing plant tends to form its new tuber at an average depth of 1 cm. This adjustment is effected by variation in length of the tuber stalk.

4. A new method of vegetative reproduction is described for *Phylloglossum.* This consists in regeneration from leaves, injured or detached by accidental causes.

5. In regeneration there is first produced an adventitious cell mass, at or near the proximal end of the leaf and arising from the abaxial surface. This cell mass is regarded as the protocorm.

6. From a growing-point differentiated on the cell mass a tuber develops which resembles that formed by the normal plant.

7. Reasons are advanced for regarding the protocorm and tuber as two distinct and independent structures.

8. The results of the investigation emphasize the biological value of the tuber, and morphological interpretations, based on anatomical evidence only, should be accepted with caution.

I am greatly indebted to Professor W. H. Lang, F.R.S., for his kindness in seeing this paper through the press.

Botanical Department,
University of Adelaide.

Literature Cited.

Bower, F. O. (1885): On the Development and Morphology of *Phylloglossum Drummondii.* Phil. Trans. Roy. Soc., London, vol. clxxvi, pp. 665–78.

——————— (1908): The Origin of a Land Flora.

——————— (1914): Address to the Botanical Section. Brit. Ass. Report, Australia, 1914, pp. 560–72.

Goebel, K. (1900): Organography of Plants. Part I. General Organography.

——————— (1905): Organography of Plants. Part II. Special Organography.

Holloway, J. E. (1914): Preliminary Note on the Protocorm of *Lycopodium laterale,* R. Br. Trans. and Proc. N.Z. Inst., vol. xlvii, pp. 73–5.

——————— (1915): Studies in the New Zealand Species of *Lycopodium.* Part I. Ibid., vol. xlviii, pp. 253–303.

——————— (1916): Studies in the New Zealand Species of *Lycopodium.* Part II. Methods of Vegetative Reproduction. Ibid., vol. xlix, pp. 80–93.

LANG, W. H. (1915): Address to the Botanical Section. Brit. Ass. Report, Manchester, 1915, pp. 701–18.

OSBORN, T. G. B. (1918): On the Habitat and Method of Occurrence in South Australia of Two Genera of Lycopods hitherto unrecorded for the State. Trans. Roy. Soc., S. Aus., vol. xlii, pp. 1–12.

ROBERTSON, A. (1906): The Droppers of *Tulipa* and *Erythronium*. Ann. Bot., vol. xx, pp. 429–40.

SAMPSON, K. (1916): The Morphology of *Phylloglossum Drummondii*, Kunze. Ann. Bot., vol. xxx, pp. 314–31.

———— (1916): Note on a Sporeling of *Phylloglossum* attached to a Prothallus. Ibid., pp. 605–7.

THOMAS, A. P. W. (1902): Preliminary Account of the Prothallium of *Phylloglossum*. Proc. Roy. Soc., London, vol. lxix, pp. 285–91.

WERNHAM, H. F. (1910): The Morphology of *Phylloglossum Drummondii*. Ann. Bot., vol. xxiv, pp. 335–47.

EXPLANATION OF PLATE XXVIII.

Illustrating Mr. Osborn's paper on Some Observations on the Tuber of *Phylloglossum*.

All figures × 20.

c.m. = cell mass; *g.p.* = growing-point; *t.s.* = tuber stalk; *v.b.* = vascular bundle of detached leaf; *v.s.* = 'vascular supply' in tuber stalk.

Photo 1. Transverse section of leaf showing considerable cell mass. A growing-point has begun to differentiate.

Photo 2. Transverse section of leaf showing cell mass. The proliferation of individual epidermal cells is shown on left. Two growing-points are differentiating, and the cell mass between them shows a continuous anticlinal wall for several cell rows. Some rhizoids (*r.h.*) have developed on left side.

Photo 3. Longitudinal section (somewhat oblique) of leaf found in field (Text-fig. 10), showing differentiation of growing-point on cell mass. The greater part of this is of epidermal origin, but secondary divisions have occurred in two mesophyll layers.

Photo 4. Longitudinal section of leaf, showing large cell mass and unusually marked secondary development in mesophyll. Note the injured surface of this leaf is oblique.

Photo 5. Longitudinal section of leaf with small cell mass and stalk of tuber. The essentially epidermal origin of the adventitious structure formed in regeneration is seen.

Photo 6. Transverse section of leaf with irregularly lobed cell mass. The larger lobe has formed a growing-point which has invaginated and lies at the base of a short pit, the channel.

Photo 7. Longitudinal section of leaf and adventitious tuber after three months' growth. The tuber has swollen and the growing-point is situated in a chamber at the upper pole. The tuber as yet shows no differentiation, nor is there any 'vascular supply' in the stalk.

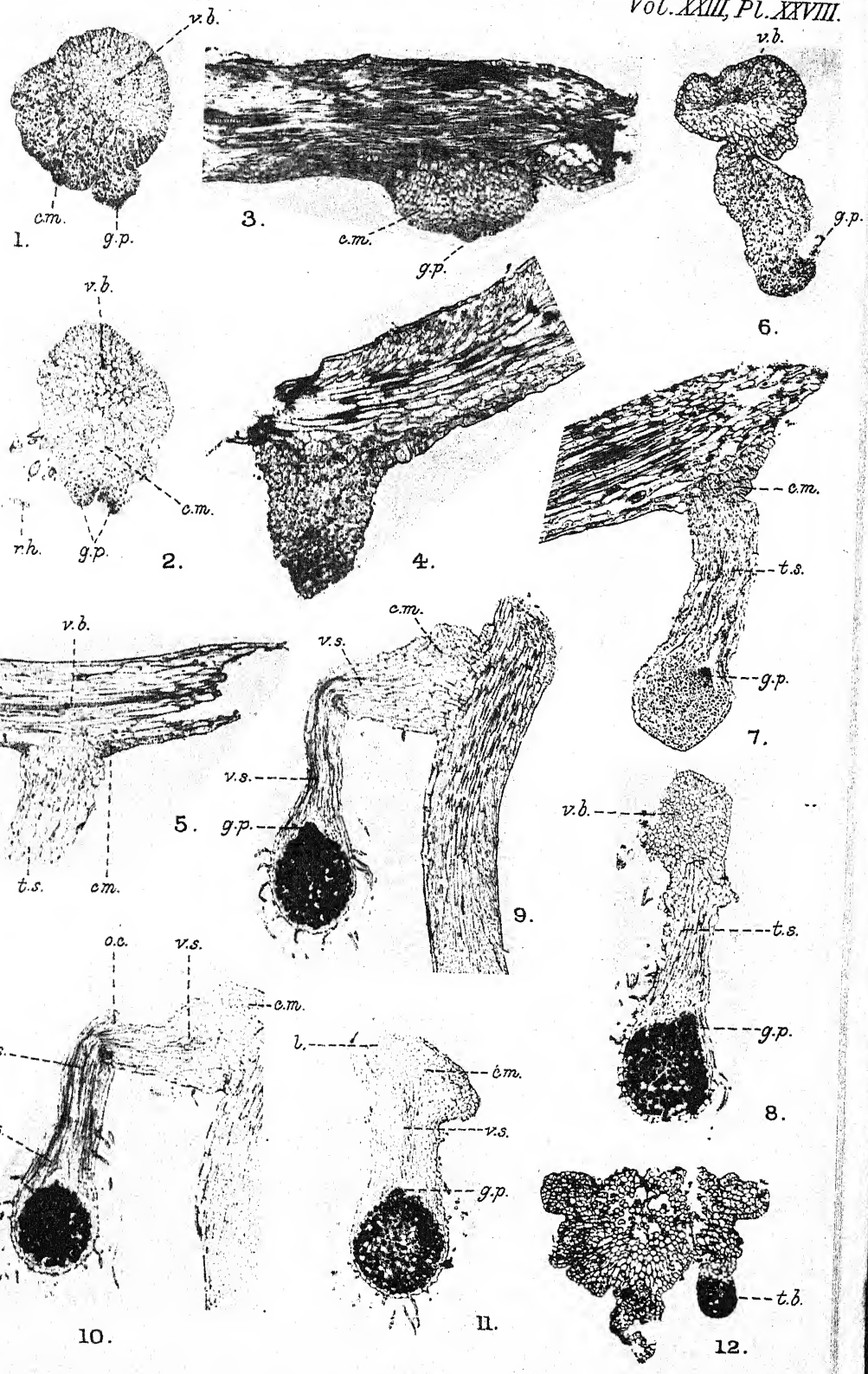
Photo 8. Transverse section of leaf bearing adventitious tuber, which is nearly fully developed. Storage tissue, tuber coat, and growing-point are all visible, and rhizoids are present on the tuber and stalk.

Photo 9. Longitudinal section of leaf and adventitious tuber at about the same stage as that in Photo 8. The bend on the tuber stalk is accidental.

Photo 10. Somewhat tangential section of same leaf as Photo 9, showing 'tracheides' of 'vascular supply' in the cell mass, and tuber stalk, with expansion at the distal end of the latter. The opening of the channel to the exterior (*o.c.*) is seen.

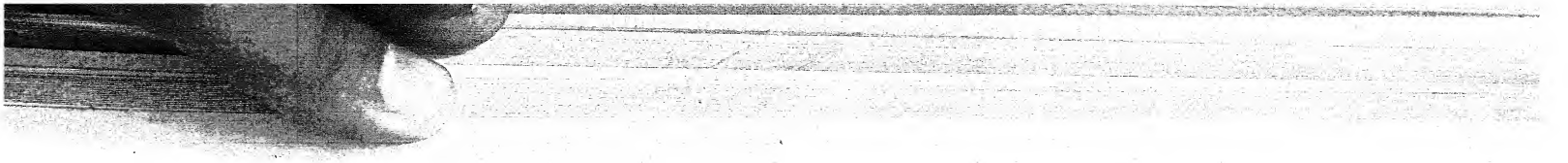
Photo 11. Longitudinal section of adventitious growth from detached leaf (Text-fig. 20) with tuber coat, bearing rhizoids, storage tissue, and growing-point. The 'leaflet' (*l.*) is seen passing out of the plane of the section on left.

Photo 12. Transverse section of leaf (Text-fig. 25) bearing many cell masses, one of which is becoming tuberous (*t.b.*) directly, without forming a definite apex.



Huth, London.

OSBORN—PHYLLOGLOSSUM.



The Temperature-coefficient of Photosynthesis: A Reply to Criticism.[1]

BY

A. MALINS SMITH, M.A. (CANTAB.).

With two Figures in the Text.

INTRODUCTION.

THREE papers have recently appeared in the 'Philippine Journal of Science', two by Brown and Heise and one by Brown alone, adversely criticizing several current conceptions with regard to the rate of carbon assimilation in plants and the influence of the factors of temperature and light upon it. In the first (1917 *a*) Brown and Heise oppose the view that the temperature-relations of this process are such that it conforms to the van't Hoff rule. In the second (1917 *b*) they oppose the view that the magnitude of carbon assimilation is directly proportional to the intensity of illumination, holding that when the light is increased by equal increments the increments of photosynthesis form a rapidly declining series. In the third (1918), by Brown alone, some attack is made upon the evidence for the conception of limiting factors.

The first of these papers is the most considerable, and is directed against the conclusions reached in a series of papers by Blackman and his co-workers, especially against the work of Matthaei (1904). The view of Brown and Heise is that carbon assimilation in plants is governed by *low* temperature-coefficients, such as have been found to characterize photochemical reaction *in vitro* (1·04 to 1·42 for a rise of 10° C.), instead of by coefficients of the magnitude of about 2·0, which are the rule for 'dark' reactions *in vitro*.

Brown and Heise have not worked upon this subject themselves and provide no new data, but they endeavour to establish their position by selection, rejection, and correction of the published results of experimental workers in this field. The intensity of their conviction that these results must be wrong when they have no data of their own to start from would be rather puzzling were it not made evident that, having grasped the generalization that all photochemical reactions have low temperature-coefficients, they have thereupon decided, quite *a priori*, that photosynthesis must really

[1] This paper forms Part XII of 'Experimental Researches upon Vegetable Assimilation and Respiration', carried out in the Botany School, Cambridge.

[Annals of Botany, Vol. XXXIII. No. CXXXII. October, 1919.]

have low coefficients also, however strong the evidence may appear to be against this. They therefore, without any misgivings, set out to explain away or discredit all measurements of carbon assimilation in plants which indicate high temperature-coefficients. There is nothing judicial or scientific in their method. Data are adopted or rejected quite apart from the amount of evidence supporting them. Sound progress can, however, only be made by shaping theories to fit facts and not *vice versa*. It has not occurred to them that though certain stages in the complex chain of reactions of carbon assimilation are certainly photochemical, yet other subsequent stages are 'dark reactions'. From this conception it follows that it would be a matter, not for *a priori* dogmatism, but for experimental investigation, whether the temperature relations of the total process were those of a 'light' or of a 'dark' reaction.

This view of the complex nature of carbon assimilation has been held by teachers and workers for many years now, and it has generally been accepted, on the available evidence, that the dark reactions govern the maximal possibilities of the system. The attack upon this view that Brown and Heise have made consists of a mass of detailed criticism of individual measurements of photosynthesis. As few readers are likely to weigh all these criticisms for themselves, it seems unfortunately necessary to point out in some detail how arbitrary and unsound they are.

Assimilation and Temperature.

Brown and Heise first discuss the work of van Amstel (1916) on *Elodea*.

Experiments of van Amstel on Elodea.

Miss van Amstel investigated the output of oxygen by *Elodea* in a current of water laden with CO_2 at a few temperatures, namely, 24°, 36·5°, and 40°.[1] She set out to measure the temperature-relations for these points, but admits that it is a difficult and complicated matter to get critical values for high temperatures. In one experiment only (Table V) did she compare the effect of the temperatures 24° and 36·5° on the same shoot of *Elodea*, and the observed increased rate indicated 1·26 as the value of K_{10}. This observation is the only low value for a temperature-coefficient of photosynthesis available in the literature, and it is of course eagerly adopted by Brown and Heise without any examination of credentials. It must first be pointed out in fairness to Miss van Amstel that she expressly disclaims that she has succeeded in establishing trustworthy ratios for these high temperatures. She says on p. 3: 'Thus the experiments, which are to be discussed here, will lead to the conclusion that in fact we did not measure the influence of temperature on the CO_2 assimilation itself, but that physical

[1] All temperature figures are in C°.

processes have exerted their limiting influence.' 'In spite of this circumstance we are publishing our results now because they may indicate to others the probable way to obtain the desired results.'

In the results presented only five are at temperatures other than 24°: three at 36°, one at 40°, one at 45°. Of those at 36° only one can be compared with assimilation at 24° because the other two were done on smaller and undefined amounts of material. Throughout this work no adequate uniformity in the amount of material employed is attained, and the values for 'apparent assimilation', under as uniform conditions as possible, vary from experiment to experiment, 0·152, 0·166, 0·168, 0·186, 0·189.

Our present interest is therefore entirely concentrated on the case where, with constant light (2,482 Hefner candles), constant CO_2 supply (350 c.c. of water containing 0·152 mg. free CO_2 per litre every $4\frac{1}{2}$ to 5 minutes), the observed output of oxygen was 0·189 mg. per minute at 24°, while it rose to 0·258 mg. at 36·5°, thus indicating a ratio which would make $K_{10} = 1·26$.

For these values to be significant it is absolutely essential that each must represent the maximal value possible at that temperature. This can only be established by producing evidence that neither light nor CO_2 supply is limiting the assimilation to a value below the appropriate specific maximum. The value of 0·189 mg. O_2 we can accept as maximal for 24° C., because raising the temperature alone considerably increased the value. But can we accept 0·258 mg. as maximal for 36·5°? Van Amstel has given no proof in her paper that 0·258 is a maximal value. There is no experiment in which the temperature was raised from 36·5° to any higher value, using the same shoot. For information as to the possibilities of assimilation at 40° we must turn to an experiment on a different shoot from that which furnished the value 0·258 mg. This second shoot gave at 40° an average assimilation of 0·242 mg. Since, however, it had already at 24° given a lower value than the first shoot, van Amstel obtained, by allowing for its lower assimilatory activity, a figure for its assimilation at 40° which was 5 per cent. higher than that of the first shoot at 36·5°. There are no data from which to calculate the probable error of van Amstel's experiments. In their absence it is safe to say that there is no proof that this rise of 5 per cent., obtained in such an indirect way, represents a real rise in assimilatory activity due to the higher temperature. Consequently it is unproven that 0·258 is the maximal value possible at a temperature of 36·5°.

Van Amstel was of opinion that she had given proof that both CO_2 supply and light were more than sufficient for the assimilation possible at any of the temperatures used in her experiments. Closer inquiry, however, may lead to a different conclusion. With regard to CO_2 supply, she trusted only to alteration of rate of flow; the concentration of CO_2 in the water-supply was the same in the whole of her experiments. The shoot

occupied the axis of the cylindrical vessel through which the water flowed and most of the flow would be along the unimpeded track at the periphery of the vessel. Now the important thing is the concentration in the more stagnant layers at the surface of the leaves and the diffusion gradient maintained from them into the leaf tissue. So when she found in one experiment that halving the total flow-rate through the cylinder did not appreciably lower the rate of photosynthesis, we cannot conclude that the concentration at the surface of the plant was seriously diminished by the reduced flow out beyond it. Experiments made at Cambridge by Mr. F. Summers on the bubbling of water-plants in a stream of tap-water supersaturated with air showed that doubling the rate of flow only increased the bubble-rate by 15 per cent. Indeed, if the flow is very fast, a further increase of 20 per cent. in rate may not increase the bubble-rate at all. Concentration of CO_2 is the only significant thing, especially in a water medium where diffusion is so sluggish, and it is a pity that the whole of van Amstel's work was done with but one concentration.

As proof that the light, 2,482 Hefner candles, was sufficient for the full assimilation at 36·5° van Amstel furnishes one experiment in which at this temperature an increase of light to 3,377 candles did not increase the assimilation (van Amstel, Table II). The value of the assimilation in this experiment (0·148 mg.) is, however, so widely different from that obtained in the later experiment at 36·5° (0·258 mg.) that, unless some explanation of the discrepancy is forthcoming, the result cannot be considered significant. For all these reasons, therefore, the figure 0·258 mg. O_2 per min. cannot be regarded as having been proved to be maximal for a temperature of 36·5°.

Further, there is the important point that no account is taken of the respiratory gas-exchange going on at the same time as photosynthesis. At low temperatures this will be small, but at high temperatures it cannot be neglected for exact work. Therefore at 36·5° the real photosynthesis value must be appreciably higher than the apparent value actually observed by just the amount of oxygen that is circulating round and round in the respiration of the moment.

It appears, therefore, that the temperature-coefficient of 1·26 is not sufficiently substantiated.

The Work of Blackman and Smith.

Brown and Heise quote experiments made by Blackman and Smith (1911) and calculate therefrom three temperature-coefficients for assimilation in *Elodea*. The first, 2·05, between 7° and 13°, agrees with the calculations of that paper. The second, 1·75, between 7° and 21°, and the third, 1·35, between 13° and 21°, were not calculated in the paper, because there was no proof that the figure obtained at 21° was the real measure of the

full temperature effect. In all probability it was the measure of a light effect. As will be seen from Fig. 7 of the paper in question, a temperature of 21° should allow of considerably more assimilation than was obtained at that temperature in either of the Expts. D or E. It was stated implicitly concerning Expt. D, and specifically of Expt. E, that the values obtained at 21° were light-limited values. Until Brown and Heise can bring forward proof that this was not the case, they are not entitled to use the results at 21° in order to calculate therefrom a coefficient for *temperature*.

It appears, therefore, from a reconsideration of the work of van Amstel and of Blackman and Smith that of the five coefficients given in Brown and Heise's Table III, four are worthless because they are calculated from results in regard to which there is no proof that temperature was exerting its maximum effect. On the contrary, there is every probability that some other factor was limiting the intensity of the assimilation. The remaining coefficient, 2·05, is perfectly sound and is of a magnitude which agrees with the van 't Hoff rule.

BROWN AND HEISE'S TABLE III.

Range of Temperature.	*Coefficient.*	*Calculated from data of*
7–13°	2·05	Blackman and Smith, p. 402
7–21°	1·75	,, pp. 400, 401
13–21°	1·35	,,
24–36·5°	1·28	van Amstel
36·5–40°	1·25	,,

The Work of Kreusler.

Kreusler (1887) worked with one shoot of *Rubus*, kept in his chamber for three weeks and exposed to a different temperature every day. Matthaei (1904) showed that the activity of the shoot is declining day after day and that the material soon becomes quite unsound, so that it is impossible to analyse out pure temperature effects from it. At the end the shoot makes little response to change of temperature, while at the beginning it may make a large response. Brown and Heise, though aware of this criticism, welcome the results of the abnormal material as being nearer to their ideal of 1·04 for the value of the temperature-coefficient. It hardly seems necessary to go further into this line of evidence.

The Work of Matthaei on Cherry-laurel.

The work of Matthaei (1904) on Cherry-laurel has been the chief experimental groundwork for the calculation of temperature-coefficients for carbon assimilation and has formed the chief support of the statement that such temperature-coefficients are in agreement with the van 't Hoff rule for ordinary chemical processes *in vitro*. Brown and Heise make a detailed attack on Matthaei's work, and it will therefore be necessary to consider somewhat fully their criticisms.

The method of Matthaei was to adopt only those numbers as measures of the influence of the temperature-factor which were obtained under conditions in which the temperature was shown to be limiting and the other factors in considerable excess. Working with one fixed light and a series of increasing temperatures, she found for that series a curve of the well-known inflected form with a rising limb for the lower temperatures, followed by a horizontal limb for the higher temperatures. Starting all over again with a more intense light, this finding repeated itself with the inflexion point

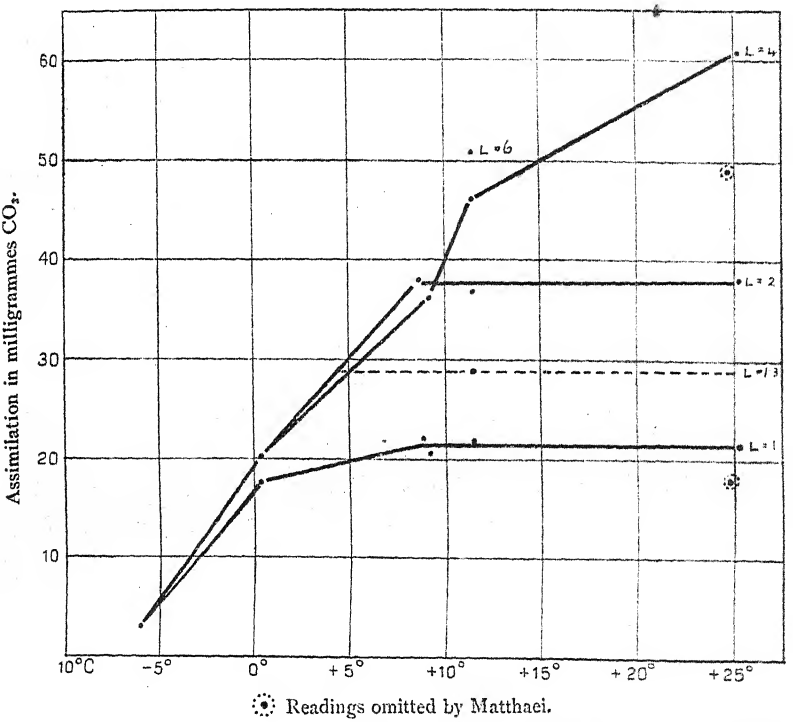


FIG. 1. This figure is redrawn from Matthaei's Fig. 3, rectifying a mistake and adding, from her tables, two omissions.

at a higher temperature, and similarly with still more intense lights, the collection of curves being shown in Fig. 1.

Her interpretation is that the rising limb is a true measure of temperature effect, while the horizontal limb is limited by light and merely expresses the light intensity, uninfluenced by temperature. Now, since the object of Brown and Heise is to show that assimilation is very little affected by temperature, they proceed on the general plan of casting doubts upon the soundness of the numbers in the rising limb of the curve (and upon these only) by appealing to seasonal changes, experimental errors, or other unsupported hypotheses. They are then left with merely a set of horizontal

limbs, one for each intensity of light used by Matthaei. As each horizontal limb does cover assimilation over a fair range of temperature, they thus reach the desired conclusion that temperature has practically no effect upon assimilation.

We must now consider in some detail the criticisms levelled at the rising limbs of each of Matthaei's four curves, which were carried out with 1, 2, 4, and 8 arbitrary units of light respectively. The first of these curves sets forth the results of twenty-one experiments ranging from $-6°$ to $+33°$, all in unit intensity of light. The curve is of very definite form, and it is clear from it that the results obtained at temperatures below 3° increase in proportion to increasing temperature, while the remainder of the results show the limiting effect of unit light. The eight experiments on the rising part of the curves are in direct opposition to Brown and Heise's contention, and they therefore simply rule them out of the discussion by announcing their intention of confining their consideration to values obtained between $+3°$ and 33°. This method of arbitrarily choosing a lower limit (3°) which will be convenient for their theory is felt by them to be too summary a procedure to pass unsupported by any argument. They therefore say, 'The part of the curve below 3° shows very much higher temperature-coefficients than would be called for according to the van't Hoff principle. At these temperatures many plant processes are just coming into activity. Under such conditions it would not be surprising to find that a general ratio would not hold for any particular function.'

To this it may be replied that results below 3° with weak light are perfectly consistent with results above 3° in somewhat stronger light when treated from the point of view of Matthaei, Blackman (1905), Kanitz (1915), and many others who accept the application of the van't Hoff rule. This is, therefore, in favour of their outlook and against the view of Brown and Heise, who have to assume a sudden discontinuity of principles at this temperature, passing from big temperature-coefficients below it to very small ones above it.

Brown and Heise then pass to Matthaei's second curve, expressing the results at different temperatures with light of twice unit intensity. It will be seen that the general form of the curve is the same as that for unit light just discussed. The important point is that though double light may produce increased assimilation, as pointed out by Brown and Heise, *yet it only does so when the temperature is raised. So long as the temperature remains low, no amount of light increase causes increased assimilation.* This fact, unchallenged, would be fatal to Brown and Heise's whole contention. They deal first of all with Matthaei's proof that at temp. 0·4° doubling the light does not increase the assimilation, while at temperatures 9°, 11°, and 25° the assimilation is thereby almost doubled. Their comment is as follows:

'The temperature is below that which we are considering. It may be

said, however, that with unit intensity of light the result at 0·4° is very similar to those obtained at all higher temperatures, and so is greater than would be expected from the more complete results recorded in Table I and plotted in Fig. 1. This figure shows an increase in assimilation of considerably more than 50 per cent. between 0·4° and 3·6°. Doubling the intensity of light did, however, increase this apparently too high result in Table II for unit intensity of light at 0·4°.'

This passage is obscure, and has been rendered more so by carelessness in the citation of tables. Table I should evidently be Table V and Table II should be Table VI. In Matthaei's paper these results are incorporated in Figs. 2 and 3 respectively.

The first sentence of their comment is a repetition of their evasion of data which do not suit them. The statement that the result in Matthaei's Fig. 3 (Fig. 1 of this paper) for c·4° is greater than would be expected from the more complete results in Matthaei's Fig. 2 is very curious, for comparison will show that the values from the two curves are exactly the same. Apparently Brown and Heise suppose that the figure for 0·4° should in every series of experiments be the same proportion of the result for 3·6° as it is in Matthaei's Fig. 2. A moment's consideration will show the improbability of such a supposition. The value for the higher temperatures shown in Matthaei's Fig. 3 (Fig. 1 of this paper) for unit light is depressed because of a lowered seasonal activity of the leaves. This phenomenon is perfectly familiar to any one who has worked with either land or water plants, and, though its causes are obscure, it is best expressed in Matthaei's words: 'It appears as if in the sluggish condition of the leaves more light were necessary to do the same amount of work.' The assimilation for unit light is therefore lower in Matthaei's Fig. 3 than in her Fig. 2. But at 0·4°, where, according to all the evidence, light is not the limiting factor, but temperature, there is no reason why the assimilation should be depressed from its usual temperature value. The result at 0·4° and light intensity one unit is therefore not too high, but, as has already been remarked, exactly the same and confirmatory of the result in Matthaei's Fig. 2. The result at the same temperature and light intensity *two* units is the same within the probable error of the experiment, and this constitutes a proof that a temperature of 0·4° is the limiting factor, for results of another order altogether are obtained when temperature is not limiting, as is shown by the three concordant experiments at 9°, 11°, and 25°.

It is well established, therefore, that with light intensity of two units a temperature of 0·4° is limiting and *a fortiori* that lower temperatures are limiting also. This is important, for it cannot be expected that in every fresh series of experiments with more intense light the proof that these lower temperatures are limiting should again be repeated. When, therefore, Matthaei comes to the experiments with light intensity of four units she is

perfectly justified in adopting these already well-established values at which temperature is limiting as part of her curve. This must be said, because Brown and Heise deal with the five experiments with light intensity of four units as an isolated set of experiments with no relation to previous values or results. These five experiments are part of the whole body of results and must be viewed in the light of them. They yield four separate temperature values, those for 9·2°, 11·4°, 24·8°, and 25·2°. These values, as plotted by Brown and Heise, seem to exhibit inconsistency, but let us look at them in the light of previous results. The curve for four units of light must be allowed to have associated with it the temperature-values well established in the previous series, e.g. those at −6° and at 0·4°. When these are added to the four values of the four-unit series itself there are obtained a series of six results which can only be consistently explained in the way adopted by Matthaei, namely, by the omission of the result for 24·8°, given by a leaf believed to be abnormal. We are not confined to the two alternatives offered by Brown and Heise, who say: 'If the figures for 11·4° and 24·8° are correct, there is very little or no rise in the rate of assimilation; if the results for 9·2° and 25·2° are reliable, there is a considerable rise.' They conclude that 'the results are such that no reliable conclusions can be drawn from them'. Even if only the four values obtained at four units light are considered there is a third explanation, the one adopted by Matthaei, viz. that the results at 9·2°, 11·4°, and 25·2° are reliable and the one at 24·8° irregular. Reinforced by the values at −6° and 0·4° her interpretation gives a consistent explanation of five out of six results, whereas the only conclusion from Brown and Heise's theory is that the whole series is valueless, a very unlikely hypothesis. Matthaei's treatment of the data is substantiated by her pointing out that the one value which is too low (at 24·8°) is given by a leaf that in a previous experiment had given an unusually low result for a less intense illumination. It is worth while to calculate the ratio for this particular leaf and compare it with the ratio for normal leaves. The ratio $\frac{\text{assimilation at 4 units light}}{\text{assimilation at unit light}}$ for the leaf in question $= \frac{485}{180} = 2{\cdot}70$. The same ratio for the normal leaf is $\frac{610}{220} = 2{\cdot}77$. The closeness of these two ratios supports Matthaei's view that the experimental result at 24·8° is due to the lower assimilatory power of the leaf in question. Nor is this view upset by the fact that at unit light the results from the two types of leaves are close enough to be within the limits of experimental error.

It is necessary here to consider the peculiar views held by Brown and Heise as to experimental error. They say 'the percentage of increase between the figure for 9·2° and the one for 11·4°, and four units of light, is, moreover, within the possible limits of experimental error, indicated in our Table V, Expts. 13 and 14'.

Now Expts. 13 and 14 of Brown and Heise's Table V (Matthaei's Fig. 2) differ by 0·00088. It is therefore not true to say that 0·00100, the difference between the experiments at 9·2° and 11·4° with four units of light, is within the previous extreme limits of error. It is outside those limits. Moreover, that error in itself was an extreme error and one which by the laws of chance would occur extremely rarely. This is made perfectly clear when one sets out precisely the probable error of the long series of fourteen experiments with light of unit intensity, ranging from $-6°$ to $+33·1°$.

The set of fourteen experiments gives a mean value of 0·0029 and a standard deviation of 0·000235, so that the probable error of a single experiment works out at $\pm 0·000158$.

That the small deviations throughout the series are devoid of any significance is shown by considering them in three groups: (*a*) the five coldest observations, (*b*) the four medium observations, and (*c*) the five hottest observations. The probable error between the mean of a group of four and the mean of a group of five is, according to the formula kindly given me by Mr. G. Udny Yule, $\pm 0·000158 \times \sqrt{\frac{1}{4}+\frac{1}{5}} = \pm 0·00011$. As the actual means of the three groups respectively are (*a*) 0·00286, (*b*) 0·00292, (*c*) 0·00288, the differences between these means are found to be actually only about half the probable error.

Brown and Heise go against all the probabilities in saying that such a large difference between two values as 0·0010 is not significant and is to be attributed to experimental error, for the unlikely deviation of $\pm 0·00044$ only occurred once.

Having claimed 0·00100, i. e. $\pm 0·00050$, as within the limits of experimental error, Brown and Heise immediately go on to claim a much smaller difference, i. e. $\pm 0·00020$ (the difference between the results for light intensities of four and six units at 11·4°), as a real light effect, not due to experimental error, indicating that further increase of light would increase still further the assimilation. This is surely merely juggling with figures in the interests of a theory. As a matter of fact, Matthaei is interpreting the results according to the probabilities, considering the general form of all her previous curves and considering how little wider this variation is than the probable error of her previous experiments, in claiming that these two results are the same within the limits of experimental error.

Brown and Heise next turn their attention to the results embodied in Matthaei's Fig. 4. These are the results of eleven experiments with eight units of light at temperatures of 11° (one expt.), 25·4° (2 expts.), 32·1° (2 expts.), 38·3° (2 expts.), 40·9° (2 expts.), and 42·9° (2 expts.). With the exception of the two highest temperatures, over 40°, these results, like those of previous sets with less light, present an initial rising curve, followed by a flat top. The difference between the results at 11° and 25·4° here is too great to be attributed by Brown and Heise to experimental error, yet, as it is necessary

for their theory to throw doubt upon the result at 11°, they have recourse to another suggestion, which I hope to show is equally improbable. 'The difference (between the result at 11° and those at 25·4° and 32·1°) can readily be explained as due to differences in the seasonal activity of the leaves,' say Brown and Heise. Now the experiment at 11° was done on Mar. 4, the experiments at 25·4° on Jan. 30 and 31, and the experiments at 32·1° on Feb. 4 and 5. In discussing the change in the seasonal activity of the leaves, Matthaei, who along with Blackman and independently had worked with Cherry-laurel leaves for some years, states that the change in the seasonal activity of the leaves occurred comparatively suddenly at the beginning of April, with the onset of warmer weather, and thus long after Mar. 4. She found in 1901 and 1902 a high assimilatory activity through March, and from her experience she felt justified in regarding this experiment of early March as belonging to the same category, as regards assimilatory activity, as the experiments at the beginning of February. Against this testimony it will need more than the unsupported assertion of Brown and Heise, who are observers in a tropical climate and have never carried out a single experiment on a Cherry-laurel leaf, that they can readily explain the difference between the result at 11° (Mar. 4) and the result at 25·4° (Jan. 31) as due to difference in the seasonal activity of the leaves. We may conclude that these experiments agree with all the previous ones and establish that with sufficient light the values put forward as characteristic of −6°, 0·4°, 9·2°, and 11° are limited by the temperature and that the true effect of increase of temperature is a rapid rise in assimilation.

Finally, Brown and Heise consider the experiments at high temperatures with very intense light and with thermo-electric records of the internal temperature of the leaf. From these experiments Matthaei obtained a rapidly rising curve. As, however, in the first three experiments both light and temperature were increased, it was necessary for Matthaei's hypothesis that she should furnish evidence that the temperature and not the light was the controlling factor. This she did by comparing these experiments with those of a former series in which in each case a larger amount of assimilation was obtained with a less intense light. The inference was drawn that the lower values in the present series were due to the limiting effect of temperature. Brown and Heise's criticism is that Matthaei's comparison was made between two sets of leaves plucked at two different seasons and therefore differing in assimilatory activity. They say that the results in the second series are lower than would be expected from the temperature of these experiments, judging from the results of the first series, and that the only possible conclusion that can be drawn from a comparison of these experiments is that the leaves used in April (the second series) were less active than those used in March (the first series). Now I propose to show that while there is indeed an error in Matthaei's reasoning, yet we

are by no means confined to the barren conclusion suggested by Brown and Heise. It is obvious that the leaves in the second series were less active than those in the first series, and Matthaei pointed this out and was fully aware of it. This lessened activity implies that the less active leaf requires more light to perform the same assimilation as the more active leaf. Now, since more light is needed for the same assimilation, comparisons cannot safely be made between the more active and the less active leaves. Thus, when Matthaei says, as a result of the comparison between the less active leaf of Expt. 56 and the more active leaf of Expt. 37, that the former must be exposed to nearly twice the light necessary for the same assimilation in a normal leaf, we cannot accept her conclusion without further inquiry. For while the more active leaf performed 0·0072 assimilation with 8 units of light, the less active leaf would certainly require more light than this for the same assimilation, and we do not know exactly how much more. It is, indeed, probable that by increasing the light to 13 units (i. e. by 65 per cent.) a sufficient margin was provided, but it is not certain. It is a practical certainty that the margin was insufficient in Expt. 57, for the light used was more than three times that used in Expt. 38, and it could scarcely therefore have been limiting even for the more sluggish leaf. But even in the case of Expt. 56 it is possible to calculate approximately whether the light provided was in excess. If the experiments of Matthaei's Fig. 3, made in April with unit light, are compared with those of her Fig. 2, made in February with unit light, it is seen that on the average the activity of the more sluggish leaf is about 75 per cent. of that of the more active one, $\frac{0{\cdot}0028}{0{\cdot}0022} = 0{\cdot}77$. It follows that if the more active leaves perform 0·0072 assimilation with 8 units of light the less active ones will require $\frac{4}{3} \times \frac{8}{1} =$ 10·7 units for the same assimilation. Therefore 13 units of light is in excess and cannot be the factor limiting the assimilation of Expt. 56 to 0·00705. Thus Matthaei's assumption is justified.

A similar calculation will show that the margin in Expt. 57 compared with Expt. 38 is so large that full allowance can be made for the lessened activity of the leaves and yet the light must be in excess and therefore the temperature limiting. Though no exact calculation can be made for Expt. 58 compared with Expt. 50, yet the probability again is that the light is in excess. This is confirmed by the result of Expt. 59, in which, though the light is not increased, the assimilation (both the initial and the average values) is greater.

This increase is therefore obviously due to the rise in temperature, showing that at 30·5° C. the temperature was the limiting factor. In this case the proof does not depend upon the comparison of leaves of differing activities at all. This fact has been dealt with by Brown and Heise as follows:

'Expt. 59, at a temperature of 37·5° and light intensity of 45 units,

shows a marked increase in the rate of assimilation over that shown in Expt. 58 with the same light and a temperature of 30·5°. The rate of assimilation at 37·5° fell off very rapidly with successive readings. This shows that at this temperature there are complicating side-reactions of considerable magnitude, so that it is not to be expected that a photochemical ratio would hold. The importance of side-reactions will be shown in another connexion. Moreover, one experiment under extreme conditions cannot be regarded as reliable when we consider the magnitude of the experimental error with medium temperatures. For the above reasons we have thought it best not to attempt to draw any conclusion from the experiment at the temperature of 37·5°. It is interesting to note that Expts. 42 and 43 (Matthaei, Table VII), with light intensity of eight units and temperature of 38·3°, do not show the decrease in the rate of assimilation that is seen in Expt. 59.'

Their first sentence recognizes a fact which they are quite unable to explain, as they will not admit the obvious solution that temperature, the only factor altered, was limiting in Expt. 58. The fact that assimilation fell with time in Expt. 59 makes their difficulty greater, for obviously it is the higher values which most nearly represent the true assimilatory activity. Hypothetical side-reactions, therefore, do not help them. Their statement about experimental error is incorrect. The difference of the first readings in Expts. 58 and 59 is 0·00080, i. e. five times the probable error, which has been shown to be ±0·00016, so that it cannot be held to be insignificant. Nor is this experiment an isolated one. It is part of a concordant series. Nor, indeed, is the temperature condition of 37·5 an 'extreme' condition. After casting up this cloud of unjustified innuendo the authors can only say that they find it best to leave the experiment out of account altogether.

The Work of Blackman and Matthaei.

Blackman and Matthaei (1905) have shown that the temperature-coefficient of *Helianthus* is even greater than that for Cherry-laurel and is probably about 2·5. They base their temperature-curve upon the results of four experiments, in each of which definite proof is given that the temperature was the controlling factor. Brown and Heise's comment on these four experiments is as follows: 'It is not evident why the changes in the rate of assimilation cannot be explained as due to variation in light intensity, especially since it is evident from their individual experiments that fluctuations in light intensity are accompanied by marked changes in assimilation.' It will scarcely be believed, after this comment, that in each of the four experiments quoted Blackman and Matthaei gave specific proof that increase of light did not increase the assimilation at that temperature. Since, however, this does not seem to have been made clear to Brown and Heise, it will be necessary to study each of the experiments mentioned.

Take first Blackman and Matthaei's Expt. X. The experimenters' statement is as follows:

'For the first four readings the temperature was kept down to about 18°, and again in the last two the temperature was the same. In all these readings except the first, which was low, due to the extremely overcast leaden sky, the assimilation numbers are remarkably uniform, 0·0089, 0·0090, 0·0089, 0·0089, 0·0092; while the light varied up and down, being especially brighter in the last two readings. This can only be interpreted as being due to the fact that the assimilation is limited by the temperature, which has been kept steady throughout. Striking confirmation of this is obtained by raising the temperature for the fifth reading. *The sky was no lighter than before*, but yet, on the temperature being brought up to 30·5°, the assimilation at once doubled, becoming 0·0163.'

In Expt. XI it is shown that the same assimilation value is obtained at the sixth reading as at the first, although the light was much brighter at the sixth reading. This can only be due to the controlling influence of temperature, as claimed by Blackman and Matthaei.

In Expt. XVI also, two readings were obtained at a temperature of 30°, in the second of which a considerable proportion of the illumination was cut off by a wooden tube, yet the assimilation did not fall, proving that temperature and not light was the limiting factor.

When there are provided these definite proofs in each experiment that the temperature was the controlling factor, of what scientific avail is Brown and Heise's statement that they would prefer to attribute all the changes to variations in illumination?

In their final discussion of the temperature-coefficient of assimilation Brown and Heise have attempted to show that its value is $1{\cdot}04 \pm 0{\cdot}03$, i. e. that assimilation is practically unaffected by temperature. They claim that this value is supported by the results of the investigators named in their Table I.

Name of Investigator.	*Name of Plant used.*	*Coefficient calculated by Brown and Heise.*
Matthaei	Cherry-laurel	1·0 +
Prjanischnikow	*Typha*	1·0 +
Kreusler	*Rubus*	1·16
Blackman and Smith	*Elodea*	1·35
van Amstel	*Elodea*	1·26

The results of all these workers have now been reviewed in this paper with the exception of those of Prjanischnikow (1876), which are not sufficiently accurate in method to merit discussion in this connexion, and it has been shown that the only accurate and trustworthy values for the coefficient are those of Matthaei on Cherry-laurel, which is 2·1; of Blackman and Smith on *Elodea*, which is 2·05; and of Blackman and Matthaei on *Helianthus*, which is not definitely given, but is probably about 2·5. All

these values are in agreement with the van 't Hoff rule for 'dark' reactions and are much higher than photochemical coefficients. Recently Osterhout and Haas (1919) have obtained a coefficient of 1·81 for *Ulva*, which is nearer to the values characteristic of 'dark' reactions than to those usual in 'light' reactions. Even this figure, however, is probably not high enough, for in the very brief details given of their experiments in the low CO_2 tension of sea-water they provide no evidence against the natural view that low CO_2 tension, based on slow dissociation, and diffusion were limiting their observed value at 37° to something lower than would have been obtained had temperature been exerting its maximum effect.

It was stated in the introduction to this paper that carbon assimilation is a complex process involving both 'light' and 'dark' reactions, and that the 'dark' reactions govern the maximal possibilities of the system. The survey since made of the work done on this problem has shown that the reliable experimental evidence is all in favour of the idea that the velocity of the whole process is determined by some reaction having a high temperature-coefficient, and not a low coefficient such as is characteristic of pure photochemical reactions.

Assimilation and Light Intensity.

In their second paper Brown and Heise (1917 *b*) maintain that the relation between light and assimilation is not one of direct proportionality, as has been held broadly by Blackman and his co-workers. They state that the relation is such that from each increase in light intensity there results a progressively smaller increase in the velocity of assimilation. This contention is based upon the experiments of Matthaei (1904), Pantanelli (1903), Reinke (1883), and Timiriazeff (1889). Brown and Heise themselves state that Timiriazeff's results are based upon faulty experimentation. They do not, therefore, put these results forward as independent evidence for their thesis. There is thus no need for any discussion of Timiriazeff's results here. The results of the other workers as interpreted by Brown and Heise need, however, some consideration.

The Work of Matthaei on Cherry-laurel.

In Fig. 3 of their first paper Brown and Heise have drawn a curve supposed to represent the relation of light intensity and carbon dioxide assimilation in Cherry-laurel based on Matthaei's data. It is clear that such a curve ought to consist of the results of experiments in which light has been definitely proved to be the limiting factor. Now of the ten values which make up this curve it can be asserted of only three that there is proof that they are light-limited values obtained from normal leaves. The value 0·0022 with unit light is light-limited, as is proved by increasing the light to two units, resulting in a large increase of assimilation at the same temperatures. The value 0·0038 with two units of light is light-limited, as is shown

by the fact that an increase of light to four units increases the assimilation at the same temperatures. The value 0·0061 at 25° and L. In. = 4 is light-limited, as it is much lower than is obtained at the same temperature with greatly increased light (cf. assimilation 0·0101 at 23·7° and L. In. = 26, Expt. LVII) from leaves at the same season of the year. On the other hand, 0·00365 at 9·2° and L. In. = 4 is quite clearly limited by the temperature, since all the remaining values for the same light at higher temperatures are considerably greater. The value 0·00485 is obtained from an abnormal leaf (see previous argument, p. 525). The value 0·00465 at 11·4° is limited by temperature, since an increase to temp. 25·2° is followed by a considerable increase of assimilation at the same illumination. The value 0·00505 with L. In. = 6 and temp. 11·4° is limited by the temperature, as it lies, within the limits of probable error, upon the temperature-curve. The value 0·0063 at 25·2° does not exist. It is an erroneous addition on the part of Matthaei to her summary table and does not appear in her full tables or in her curve.

The remaining values, 0·0070 at 15° and L. In. = 13; 0·0101 at 23·7° and L. In. = 26; and 0·00136 at 30·5° and L. In. = 45. are all values limited by the temperature for reasons already fully set forth. Thus Brown and Heise's curve is partly a light-curve, but principally a temperature-curve, and therefore does not show the relation between light intensity and assimilation as asserted by them.

The Work of Pantanelli.

In the paper by Blackman and Smith (1911) it was shown that Pantanelli's curve was of the usual compound nature, only the first part showing a true light effect, the later part showing probably the effect of limiting CO_2 supply or possibly of temperature. Brown and Heise reject this explanation of Pantanelli's curve and draw the curve again in such a way that they can interpret it as giving support to their thesis. Now in their exposition, in addition to minor matters of questionable soundness which are here omitted for lack of space, Brown and Heise have arbitrarily omitted from their curves all values above unit light. Yet Pantanelli conducted experiments at light intensities of 4, 9, 16, 25, 36, 49, and 64 units. It has been shown in our paper that while the values obtained in the highest intensities of light are justly omitted from the curve, giving smaller values, attributed to 'fatigue' of the chloroplast, yet the values at 4 and 9 units form an important part of the whole evidence. If it be asked why Brown and Heise have omitted, without any explanation or reference to the fact, all the results in light intensities greater than unity, the only answer at all possible is that these results are entirely fatal to their theory. Whether the stationary results at medium intensities of light or the depressed results at higher intensities are considered, there is here a long range of experiments in which the assimilation *does not increase at all* and the values cannot be

represented by a rising curve of any kind, logarithmic or other, but simply by a straight horizontal line or indeed later a falling curve. Just as Brown and Heise, in the interests of their *a priori* theory, ignored the low temperature results of Matthaei, so here, and for the same reason, they ignore the results of Pantanelli at the higher intensities of light.

The Work of Reinke.

Brown and Heise have used Expt. VIII of Reinke as the chief basis of the argument in which they attempt to obtain support for their theory from Reinke's work. The curve of Expt. VIII, however, can be interpreted, with greater probability, as one which shows the action of two limiting

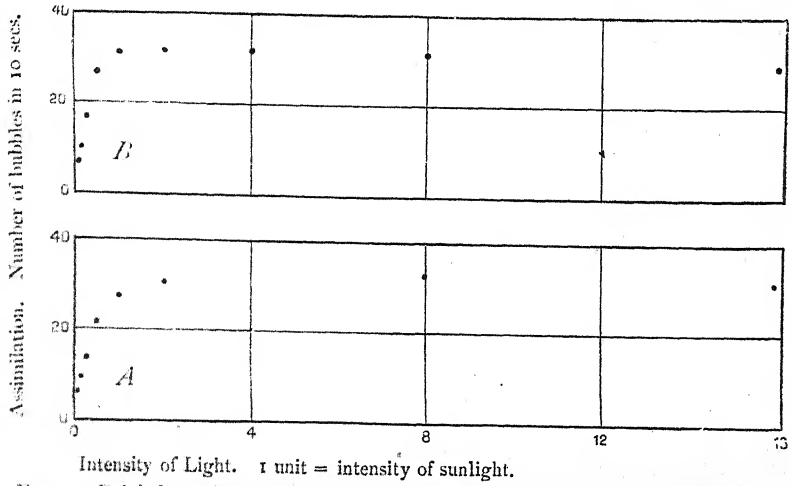


FIG. 2. Reinke's results. Curve A. Average of all results in Tables I to VIII. B. Average of all results except those obtained after the plant had been once exposed to 16 units of light. Reading 60? with unit light in Expt. VI omitted from both curves.

factors, first the light and later the CO_2 supply. But Expt. VIII does not show this so conclusively as do some of the other experiments, notably Expts. I, III, and VIII. In order to avoid all theoretical bias in the interpretation of Reinke's experiments, Fig. 2, Curve A, has been drawn showing the average of *all* the experiments made by Reinke with his first arrangement of lighting, i.e. all the results shown in his Tables I to VIII. There can be no doubt that the curve obtained is one of the familiar type showing the action first of light as the limiting factor and later of CO_2 supply. Already at unit light the assimilation reaches its maximum and remains sensibly at that figure up to an intensity of 16 times sunlight.

Brown and Heise's Table II gives the results for all Reinke's experiments, and they say, 'These results are in agreement with the more detailed calculations given in Table I for Reinke's Table VIII'. As a matter of fact, the results in their Table II do *not* support their hypothesis. In Expts. I, III, and VII no increase is shown between unit sunlight and 16

units. In fact the only figures which give their hypothesis a semblance of support are those in Expts. IV, V, and the later portions of VIII. It will be noticed that in these cases the plant has been exposed for some time to high intensities of light, and that on being taken back into lower intensities its curve is slightly different from that of a fresh plant. It seems clear that the plant has been to some extent injuriously affected by the intense light. This supposition is supported by the fact that in Expt. VIII, where the plant is twice exposed to a light intensity of 16 units, the bubble emission gradually falls off through the experiment, so that the last row of figures is throughout the smallest of the whole series. In order to avoid this source of error Curve B, Fig. 2, has been drawn, representing the readings after ignoring all those taken after the plant has been once exposed to 16 units of light. This curve is a typical limiting factor curve and cannot possibly be interpreted as supporting the hypothesis of Brown and Heise. It is significant of the small amount of support which Brown and Heise can obtain from any of the experiments, that Curve B is scarcely more conclusively in favour of the interpretation of Blackman and Smith than is Curve A, obtained from all Reinke's readings whatsoever. In fact Brown and Heise practically admit this when they say, 'This progressive falling off in assimilation per unit of increase of light is very rapid, and it is, therefore, not surprising that with high light intensities increasing the intensity does not greatly augment the rate of assimilation', and later, 'if a direct proportionality is found in such a case (i.e. in low intensities of light) this is due to the selection of a particular range of light intensities'. In the first of these sentences they admit the fact that at higher intensities increase of light does not increase assimilation, and in the second that at the lower intensities the assimilation is proportional to the light. It only remains for it to be noted that the latter is true up to half sunlight and the former from unit sunlight to 16 units in order to make it clear that the hypothesis of two limiting factors is correct, on Brown and Heise's own admission, through the whole range of intensities used in Reinke's experiments.

Recently Boyson-Jensen (1918) has given curves showing the effect of light upon assimilation. As he points out, these curves are of a dual nature, showing first the limiting effect of light and later that of CO_2 supply. Owing, however, to the fact that individual variations were not eliminated, the results are not sufficiently exact to form a decisive test between the interpretation of Brown and Heise and that of Blackman.

Assimilation and Limiting Factors.

In a third paper W. H. Brown (1918) has made an attempt to prove that the results of Blackman and Smith (1911) on *Elodea* and *Fontinalis* can be more accurately represented by a so-called optimum curve than by the

limiting factor curve drawn in that paper. A critical glance at Brown's Fig. 1 will show that the so-called improvement in presentation put forward by Brown is merely a matter of drawing a line arbitrarily between the various experimental values, so as to produce an illusory effect of convex curvature, rather than horizontality. His proposed correction of the latter part of the curve by allowing for the temperature-coefficient is quite illegitimate, for convincing evidence was given that both the values to which Brown has applied this correction were already much below the maximal values for those temperatures. The values were, in fact, kept low by the controlling influence of some other factor, presumably light. As, therefore, they are not temperature-values at all, they cannot be subjected to a correction for temperature-coefficient. Apart from this proposed change, the difference between Brown's curve and that put forward by Blackman and Smith is insignificant. The attempt made by Brown to separate the earliest part of the curve into two individual parts is seen, when closely examined, to involve him in inconsistencies. It is not proposed, however, here to follow him into these details of interpretation, for it is not upon such details as these that the validity of the theory of limiting factors as applied to carbon assimilation depends. This theory does not depend upon any single curve, nor can it be overthrown by suggesting, as Brown has done, minor differences in the interpretation of any single curve. Thus Matthaei's supposition that in her earliest curve light was the limiting factor at all temperatures higher than 3°, did not become established from a consideration of the form of that particular curve, but from the fact that by subsequent increases of the intensity of the light she obtained *a whole series* of similar inflected curves, each one higher than the last in correspondence with the increased light. In a similar way, though not so completely, Blackman and Smith, in the paper under discussion, showed that a light of 6·0 units was limiting the assimilation in their general curve, because raising the light from 4·2 to about 6·0 units caused an increase in the rate of assimilation.

The revival by Brown of the hypothesis that there exists an 'optimum concentration of carbon dioxide', which is indicated by his drawing of Blackman and Smith's curve, has surely very little value in the light of the experimental proof that the assimilation corresponding to that optimum can be varied as desired by changing the intensity of the light used in the experiments. When such a series of curves is obtained, as was obtained by Matthaei for Cherry-laurel in increasing intensities of light, any one of these curves may perhaps be of the form preferred by Brown, i. e. such as he would call an optimum curve. But how can all the curves be optimum curves, and what is the value of the idea of an optimum which changes with every change of one of the factors conditioning the experiment?

It is not claimed that a limiting factor curve always adheres rigidly to a typical form with a sharp angle at the point of change of the limiting factor.

It is conceivable, and is indeed probable, that when, so to speak, two factors are close to the limiting value a change in the one not limiting may have some appreciable effect on assimilation. This will show itself about the inflexion of the curve where the limiting factor is changing. For example, when CO_2 is limiting, increase of temperature may cause a small increase of assimilation by increasing the rate of diffusion of the CO_2. But all minor details like these apart, the hypothesis of limiting factors rests broadly on the possibility by its means of interpreting simply and logically the greatest number of the known facts about the rate of carbon assimilation.

Finally, Brown reveals his singular lack of appreciation of the complexity of the system involved in assimilation when he asks how such a principle as that of limiting factors can hold in the phenomena of assimilation when he can see no sign of this principle at work in the action of hydrochloric acid upon marble or the solution of a gas in water.

I am glad to acknowledge the valuable assistance of Dr. F. F. Blackman, F.R.S., with whom I have discussed many of the points raised in this paper.

List of References.

Blackman, F. F. (1905): Optima and Limiting Factors. Ann. Bot., vol. xix.

Blackman and Matthaei (1905): A Quantitative Study of Carbon Dioxide Assimilation and Leaf-temperature in Natural Illumination. Proc. Roy. Soc., B., vol. lxxvi, pp. 402–60.

Blackman and Smith (1911): On Assimilation in Submerged Water-plants and its Relation to the Concentration of Carbon Dioxide and other Factors. Proc. Roy. Soc., B., vol. lxxxiii, p. 389.

Boyson-Jensen (1918): Studies on the Production of Matter in Light- and Shade-plants. Bot. Tidsskrift, 36 Bind, 4 Hefte.

Brown and Heise (1917*a*): The Application of Photochemical Temperature-coefficients to the Velocity of Carbon Dioxide Assimilation. Philippine Journ. of Science, Botany, vol. xii, p. 1.

——— (1917*b*): The Relation between Light Intensity and Carbon Dioxide Assimilation. Phil. Journ. Sci., Bot., vol. xii, p. 85.

Brown, W. H. (1918): The Theory of Limiting Factors. Phil. Journ. Sci., Bot., xiii, p. 345.

Kanitz (1915): Temperatur und Lebensvorgänge.

Kreusler (1887): Beobachtungen über die Kohlensäure-Aufnahme und -Ausgabe der Pflanzen. Landwirthschaftliche Jahrbücher, p. 711.

Matthaei (1904): On the Effect of Temperature on Carbon Dioxide Assimilation. Phil. Trans., Series B, vol. cxcvii, pp. 47–105.

Osterhout and Haas (1919): The Temperature-coefficient of Photosynthesis. Journal of General Physiology, vol. i, p. 295.

Pantanelli (1903): Abhängigkeit der Sauerstoffausscheidung belichteter Pflanzen von äusseren Bedingungen. Jahrb. f. wiss. Bot., vol. xxxix.

Prjanischnikow (1876): Bot. Jahresbericht.

Reinke (1883): Untersuchungen über die Einwirkung des Lichtes auf die Sauerstoffausscheidung der Pflanzen. Bot. Zeit., pp. 1–14.

Timiriazeff (1889): Sur le rapport entre l'intensité des radiations solaires et la décomposition de l'acide carbonique par les végétaux. Compt. Rend., 109, p. 379.

van Amstel (1916): On the Influence of Temperature on the CO_2 Assimilation of *Elodea canadensis*. Rec. Trav. Bot. Néerl., vol. xiii, pp. 1–29.